Chasing the taxidermist's tail: Using portable X-ray fluorescence spectroscopic analysis to reconstruct museum specimen data

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Faculty of Science, School of Chemistry

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February 2024

Statement of originality

This is to certify that, to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

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The convention in this discipline is to list authors in order of contribution, with largest contributor listed first. I designed the study, collected and extracted the data. The data was analysed with the support of Dr Brad Swarbrick. I then interpreted the analysis, produced all images, and prepared the manuscript. All authors contributed to the revision of the article. Permission to include the article in my thesis has been granted by all authors. The material included in this publication relates to research undertaken during my candidature and was published during my candidature.

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As supervisor, I support this statement identifying the student's contribution to the work covered in this article.

Dr Elizabeth A. Carter Date

Substantial sections of the **Abstract** from this thesis was published in Conference proceedings of the Australian Institute for the Conservation of Cultural Material (AICCM) National Conference 2023. Limited print form only. I prepared the manuscript and images for this publication. Prof Peter Lay and Dr Elizabeth Carter contributed to its revision. Permission to include the article in my thesis has been granted by all authors. The material included in this publication relates to research undertaken during my candidature and was published during my candidature.

Celia Cramer Date

As supervisor, I support this statement identifying the student's contribution to the work covered in this article.

Dr Elizabeth A. Carter Date

Contributions to experiments and editing

Scanning electron microscopic images seen in Chapter 1 were produced with the assistance of Ashalatha Indiradevi Kamalasanan Pillai.

Wet chemical experiments including the creation of experimental standards discussed in Chapter 2 were undertaken with the help and guidance of Dr Aviva Levina, School of Chemistry, The University of Sydney.

Inductively coupled plasma mass spectrometry digestions, dilutions, and data factoring discussed in Chapter 2 was completed with the assistance of Dr Nicholas Proschogo, Technical Support Services, The University of Sydney.

Chapter 6 was proofread, and copy edited by Dr Brad Swarbrick who gave advice about clear language in accordance with the Australian standards for Editing Practice (ASEP) 2013.

Chapter 6 was proofread, and copy edited by Dr Vanessa Finney who gave advice clear language in accordance with the Australian standards for Editing Practice (ASEP) 2013.

Supervisor's statement

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct. I confirm that this thesis is sufficiently well presented to be examined and does not exceed the prescribed word limit.

Dr Elizabeth A. Carter Date

Acknowledgements

This research reported in this thesis was supported by the award of a Research Training Program scholarship to the PhD Candidate

This research was funded in part by the Australian Research Council Linkage Grant LP1601761, and by the Harvard University Committee on Australian Studies.

The author acknowledges the facilities and the scientific and technical assistance of Sydney Analytical, and Sydney Microscopy and Microanalysis, core research facilities at The University of Sydney.

The author gratefully acknowledges the assistance provided and access to specimens located within the Australian Museum, Sydney, and the Chau Chak Wing Museum, The University of Sydney

To my supervisor, Dr Elizabeth Carter of The University of Sydney, words of thanks seem inadequate in acknowledging the friendship, knowledge and support you have shared with me. Grateful thanks also to Prof Peter Lay and Dr Jude Philp for such great support and generous sharing of your experience, especially in these last few months.

The author would like to gratefully acknowledge the kind support and guidance of the following people without whom, my ideas would not have become reality…Many thanks to Dr Nicholas Proschogo and Dr Aviva Levina, of The University of Sydney, who's patience and support was invaluable in designing and safely running experiments with all those lovely toxic chemicals. Dr Sarah Kelloway for her careful guidance on XRF application in the early years of the research. Dr Sandy Ingleby and Harry Parnaby of the Australian Museum who tirelessly supported my work by retrieving specimens and archival gems from the Museum's great reserves of information, and for helping with all those tricky taxonomic names and

conventions. Thank you, Dr Tony Gill from the Chau Chak Wing Museum, The University of Sydney who sat with me hour after hour during data collection, providing good humour and conversation. Ashalatha Indiradevi Kamalasanan Pillai from Sydney Microscopy and Microanalysis, The University of Sydney, who introduced me to the marvels of SEM, thank you. Many thanks to Dr Brad Swarbrick and Dr Joonsup Lee, of the KAX Group Sydney, who restrained their amusement with my confusion and helped me master their great software. Dr Kenny Travouillon who guided me in the complex world of Australian bandicoots. Dr Josh Bell of National Museum of Natural History, Smithsonian Institution for your very thoughtful response to the early drafts of the data deficit chapter and provoking me to commit to my ideas. Lee Drake for your encouragement and suggestions on developing this research into the future. Dr Mark Omura of Harvard University's Museum of Comparative Zoology, for your help delivering and orienting my presentations at Harvard for an American audience, and helping me to see how my research may be useful beyond Australia. Judith Jungles of the Peabody Museum at Harvard University, for being my guinea pig in trying out my data collection protocol and helping me to find ways to describe it.

To my colleagues who's combined efforts resulted in a body of data on Australia's specimen trade that has framed my contribution. You have supported me in academic and personal ways…. Dr Vanessa Finney of the Australian Museum for stimulating conversations, encouragement to keep writing, and making me laugh at the discomfort of being a PhD candidate. Prof Deirdre Coleman and Dr Henry Reece, University of Melbourne, for encouragement when I was feeling a bit like an imposter – couldn't have asked for a better great cheer squad! Prof Simon Ville, University of Wollongong, for your steadfast faith in me. Dr Robin Torrence, Australian Museum, for giving me the confidence to pursue publication.

Finally, to my husband Charles Cramer, my children Evelyn and Hugh Cramer who read each draft and provided useful feedback, and my daughter Gwen who provided welcome breaks and endless cups of tea.

List of publications and presentations

Publications

- Cramer, C., Carter, E.A., Swarbrick, B. Philp, J, Pay, P.A. Pursuing pademelon provenance: a pilot study using portable XRF to trace field-collection of museum mammal specimens. *Herit Sci* 2023 25 July; 11:151. https://doi.org/10.1186/s40494-023-00998-1

Video Publications

The Australian Museum Research Institute invited C Cramer to make this presentation to the general public.

- Cramer, C. Chasing the Taxidermist's Tail: Using chemistry to reconstruct specimen collection data in natural history museums [internet]. Sydney: Australian Museum Research Institute; 2021 Dec 14 [cited 2024 Feb 14]. 18:21 mins. Available from: https://australian.museum/get-involved/amri/amri-seminars-and-lectures/reconstructing-collection-data-using-chemistry/

Oral presentations

Invited presentations

- Cramer, C. 'Dissecting data deficit'. Paper presented at: Negotiating Nature: Donors, Collectors and Merchants in 19th century natural history museums symposium, Department of History, Harvard University; 2023 31 March – 1 April 2023; Boston, USA.

- Cramer, C. 'Preservatives to provenance'. Paper presented at: Negotiating Nature: Donors, Collectors and Merchants in 19th century natural history museums symposium, Department of History, Harvard University; 2023 31 March – 1 April 2023; Boston, USA.

- Interview with Lisa Pellegrino on Nightlife on ABC Radio. 2023 13 Jan

- Cramer, C. Tale of two taxidermists: Finding provenance of natural history specimens in preservative residues. Paper presented at: the Spectroscopy for Cultural Heritage Workshop, Sydney Analytical and Bruker Instruments; 2022 5-6 October; Sydney, Australia.

- Cramer, C. Chasing the taxidermist's tail: Is it possible to find the field-collector by looking at the preservative residues? Paper presented at: the Australian Museum Research Institute Student Forum; 2021 22 November; Sydney, Australia.

Contributions selected for presentation

- Cramer, C., Carter, E.A., Philp, J, Pay, P.A. Using pXRF to identify the object histories of zoological museum specimens. Paper presented at: the Australian Institute for the Conservation of Cultural Material (AICCM) National Conference; 2023 15-17 November; Canberra, Australia.

- Cramer, C., Carter, E.A., Swarbrick, B. Philp, J, Pay, P.A. Investigating the potential of NIR spectroscopy to provenance natural history museum specimens. Paper presented at: the Infrared and Raman Users Group (IRUG) 15th International Conference; 2023 26-29 September; Tokyo, Japan.

- Cramer, C., Carter, E.A., Philp, J., Lay, P. & Reece, H. Chasing the taxidermist's tail: networks of natural history. Panel session presented at: Truth telling through university museums and collections. International committee for university museums and collections conference (UMAC) 2023; 2023 28 August – 1 September; Sydney Australia.

- Cramer, C., Carter, E.A., Swarbrick, B. Philp, J, Pay, P.A. Chasing the taxidermist's tail: Investigating the potential of NIR spectroscopy to provenance natural history museum specimens. Paper presented at: the 19th National Conference of the Australian Near Infrared Spectroscopy Group (ANISG); 2022 12-14 September; Cairns, Australia.

- Cramer, C., Carter, E.A., Philp, J. Chasing the taxidermist's tail: Using spectroscopy to find specimen data. Paper presented at: the Australian Institute for the Conservation of Cultural Material (AICCM) National Conference; 2019 13-15 November; Melbourne, Australia.

Abstract

During the nineteenth century, millions of animals were collected, preserved, traded, and incorporated into natural history collections around the world. The twin cultural movements of museum creation and zoological specimen aggregation formed valuable resources for knowledge making and education. These resources continue to inform understandings of environment, biodiversity, and cultural histories.

Much of the value of museum collections is held within the contextual information associated with each specimen. Over time however, many specimens have become separated from their associated data leaving them unreliable as resources for research. Archival research has been the main tool in reconnecting museum specimens with their histories but is often constrained by gaps in the documentary records. Establishing the geographic origin of a specimen using scientific analysis typically requires the removal of a physical sample, diminishing often irreplaceable historic biological material.

This research recognises zoological specimens as human-made objects, transformed from a living animal into a scientific resource by field collectors who applied chemical treatments to prepare and preserve them. This dissertation documents a novel protocol combining of X-ray fluorescence spectroscopy, principal component analysis, and archival investigation to bridge historical gaps and re-establish specimen contextual data while preserving the integrity of the historic biological material.

For the first time, the chemical residues of preservatives and pesticides were used to connect specimens with their makers. Patterns in the data revealed new ways of recognising the treatment histories of specimens. The remnants of chemical applications were used to discriminate specimens collected by individual field collectors or collecting groups. With this new-found knowledge, archival investigation was redirected, and unprovenanced ‘no-data’ specimens reconnected with their field collection histories.

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Abbreviations and Definitions

Aepyprymnus rufescens	Rufous Bettong
Al	Aluminium
Aprosmictus erythropterus	Red winged parrot
As	Arsenic
Bi	Bismuth
Br	Bromine
Ca	Calcium
Cl	Chlorine
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic acid
Fe	Iron
Hf	Hafnium
Hg	Mercury
ICP-MS	Inductively coupled plasma – mass spectrometry
K	Potassium
kV	Kilovolt
Mn	Manganese
Mount	Preserved animal skin supported by an internal form replicating the animal in life
Ni	Nickel
Non-destructive or Non-invasive	Testing without the extraction of a physical sample form a heritage specimen
P	Phosphorus
Pb	Lead
PC	Principal Component
PCA	Principal Component Analysis
Perameles myosuros	Western barred bandicoot
Pesticide	Applied after the production of a zoological specimen with the purpose of killing insects and rodents
Pickle	Liquid for bathing animal skins immediate after removal for the carcass
Preservative	Applied at the time of skinning with the purpose of preventing putrefaction of the animal skin
pXRF	portable X-ray fluorescence spectrometer
Rb	Rubidium
RNA	Ribonucleic acid
s	Seconds
S	Sulphur
SEM	Scanning electron microscopy
Si	Silicon
Sn	Tin
Spirit specimen	An animal preserved and stored in liquid, typically ethanol-based
Spirits of wine	Liquid typically high in ethanol derived from fermentation of fruit
Sr	Strontium
Study skin	Preserved animal skin arranged to take up little space and created for assisting research
Thylogale billardierii	Tasmanian pademelon
Thylogale stigmatica	Red-legged pademelon
Thylogale thetis	Red-necked pademelon
Ti	Titanium
Tl	Thallium
W	Tungsten
XRF	X-ray fluorescence
Z	Zeta
Z weight	Atomic weight or atomic number
Zn	Zinc
α	Alpha
β	Beta
γ	Gamma
η	Eta
ι	Iota
µA	Microamps
♀	Female
♂	Male
o	Gender unknown, often because it is a juvenille

Chapter 1

1.1 Introduction

Humans have utilised animal skins in a myriad of ways (1). The seventeenth century expansion of European concepts of nature and the natural world gave rise to a period of enthusiasm for collecting animals and their skins that was far greater than any that preceded it (2). Animal skins became representatives of this natural world and were used to understand and engage with the exploration and colonisation of places beyond the European sphere (3). Privately owned collections of curiosities included the skins of unusual animals and these became social tools to express wealth and power among Europe's elite (4).

Throughout the eighteenth century, the Enlightenment movement saw interest in the natural world develop into an occupation, that of naturalist. Initially amateur enthusiasts, naturalists studied animal specimens in endeavours to order and interpret the diversity of nature (5). In the field, hunters, naturalists and skin preparators worked in concert to collect, identify and preserve specimen skins (6). These were the field collectors. In towns and cities, small mixed businesses offered preservation and stuffing services to transform animal skins brought in by field collectors into sculptures that represent the living animal (3,7,8). These taxidermists became vendors of zoological specimens (9). State-owned natural history museums emerged with large and diverse collections often created from the private collections of the previous century (10-12).

By the early nineteenth century, trade and fascination with natural history specimens was burgeoning (3,13). Naturalists and collectors, from all walks of life, gathered animal skins for "profit, glory or curiosity" (10). Socio-economic changes within colonising and colonial societies created a new group of naturalists, wealthy enough to purchase specimens, and keen

to express their newly acquired status (10). Zoological skins appeared in fashionable clothing and as decorative objects in homes (3,14,15). Zoological specimen shops and stuffing studios had become viable and even lucrative businesses (12,16). In Australia, as in other parts of the world, museums mustered field collectors and taxidermists to ensure a constant supply of animal skins for distribution to private collectors and state museums (17,18). Museums and professional naturalists focussed on collecting 'scientific' specimens. These were discriminated from decorative objects by the retention of information about the animal, and the environment from which it was extracted (19-21) i.e., the specimen's data.

1.2 What is specimen data?

Behind each zoological specimen is a web of relationships that connects people, places, objects, and nature itself (22-24). These relationships include those between the animal and its environment, the animal and the field collector, the field collector and the natural history collection (often via one or more specimen dealers), and the natural history collection and its visitors and researchers. These are 'cross-linked' by relationships between specimens brought together at the same time, either in the field, or within a museum collection or exhibition (25). These relationships are made explicit in a specimen's data.

Three pieces of information are fundamental in specimen data; specimen name, date, and location of collection from the wild (field collection) (26,27). Additionally, contextual information is often included, e.g., environmental details or names given by Indigenous peoples. In 1849, Richard Owen described the content and the importance of specimen data in the nineteenth century.

> "The exact locality in which several specimens were procured is of great importance in the determination of the laws of geographic distribution of mammalia: and not only the country, but the nature of the country, its elevation and geological character, as nearly

> as can be ascertained. Also the degree of commonness of the animal and any of its known habits, and native name" (21 p.381).

Museum zoological specimens and their data are used today to understand animals, their taxonomic relationships, and the environments they are held to represent (28-31), and are valuable resources in interpreting changes over time (20,32-34). Museum specimen data are also used to reveal changes in interactions between humans, the natural world, and the different ways by-which knowledge is made and shared over time (35-38). Susan Star reminds us that what is missing from the physical remnants of science tells us as much as what is present (39). It is in these small data "silences" (40 p.26-27) that we can find traces of past human interaction, of the agency of the players that have shaped the materiality of museum collections, and thus, our interpretation of that material into scientific observations, and cultural history narratives (41).

While individual aspects of specimen data are important to the different researchers who use them (27), the connection between the specimen and its data remains integral. 150 years after the publication of Owen's instructions for collecting specimen data, Lane explained the enduring value of museum specimen data.

> "Together, a preserved organism and its label are a scientific specimen that has great intrinsic value. Separately, the label is a piece of paper with meaningless inscriptions upon it, and the plant, spider, microbe, mushroom, or bird, though carefully preserved, is just so much dead organic matter" (4 p.536).

Specimens with no data, data gaps or data inconsistencies are often excluded from research (26,42-44). In 2020, the American National Academy of Sciences acknowledged that the exclusion of these specimens "represents lost opportunities for research and education as well as limits to return of the investments made by the funding agencies that support the acquisition

of the specimens" (45 p.244). Today, discarding such specimens and replacing them with new material from the wild is no longer an acceptable practice (31,46). Economic, social and regulatory restrictions on specimen collection, diminishing biodiversity and increased extinctions have highlighted the importance of each museum specimen (20,47-50). With it, the desire to retain and preserve each museum specimen has increased. Yet the critical treatment history information is absent from most museum specimens (50-52). Returning the scientific potential of specimens without adequate data is a significant challenge.

Fig. 1.1: 'No data' labels are not uncommon in natural history collections. While this specimen may have been collected as early as the 1830s, its label shows that by 1912 no contextual specimen data remained. Image produced with permission from the Australian Museum.

1.3 Recovering lost specimen data

Methodologies to reconstruct missing or unreliable specimen data focus primarily on documentary evidence (22). The successful reconstruction of specimen data is typically the result of tracing a trail of archival 'breadcrumbs' - receipts, bills, collector correspondence, field-notes, and biographies (53,54). Identifying early connections – those between the animal

and its collector – has always been challenging (55). Here the material evidence, the specimen itself, has offered opportunities for alternative approaches. These fall into two main groups - stylistic and chemical analysis.

1.3.1 Stylistic analysis

Investigating provenance of cultural objects via material evidence is a well-established practice in a number of disciplines (56). Researchers have used characteristic marks (intentionally or incidentally) made in the process of creating an object, as evidence of a particular individual or group of makers. By acknowledging that museum zoological specimens are human-made objects, produced for a specific, cultural purpose, then the same notion may be applied (30,50,57,58).

Stylistic aspects of specimen preparation and physical markings have been used to connect specimens with lost histories of their manufacture. Pequignot assessed three-dimensional forms used to fill a collection of mammal skins to identify their period of manufacture (50) and differentiated birds specimens prepared by specimen dealers from those prepared by museum staff by their posture (6). Gray and Renner used the bryophyte stuffing of Kākāpō (*Strigops habroptilus*) specimens to inform field-collection location (59). Internal wiring and remnant bones, investigated using radiography, have been used by Rasmussen and Collar (60), Williams and Hawks (52), Prŷs-Jones (61), Philp, *et al*. (62), and Morris (63,64) to associate specimens with taxidermists and collectors. Patchett used the shape of mounting boards and the animal's facial expressions to interpret the provenance of taxidermized tiger heads (65). Knox used the arrangement of limbs, incision patterns, and thread types to clarify the debated provenance of a collection of bird specimens (66). In the skull of a Rufous bettong specimen, Museums Victoria staff identified physical markings indicative of its capture by Australian first nations field collectors (67). Stylistic techniques rarely require any alteration to the specimen itself.

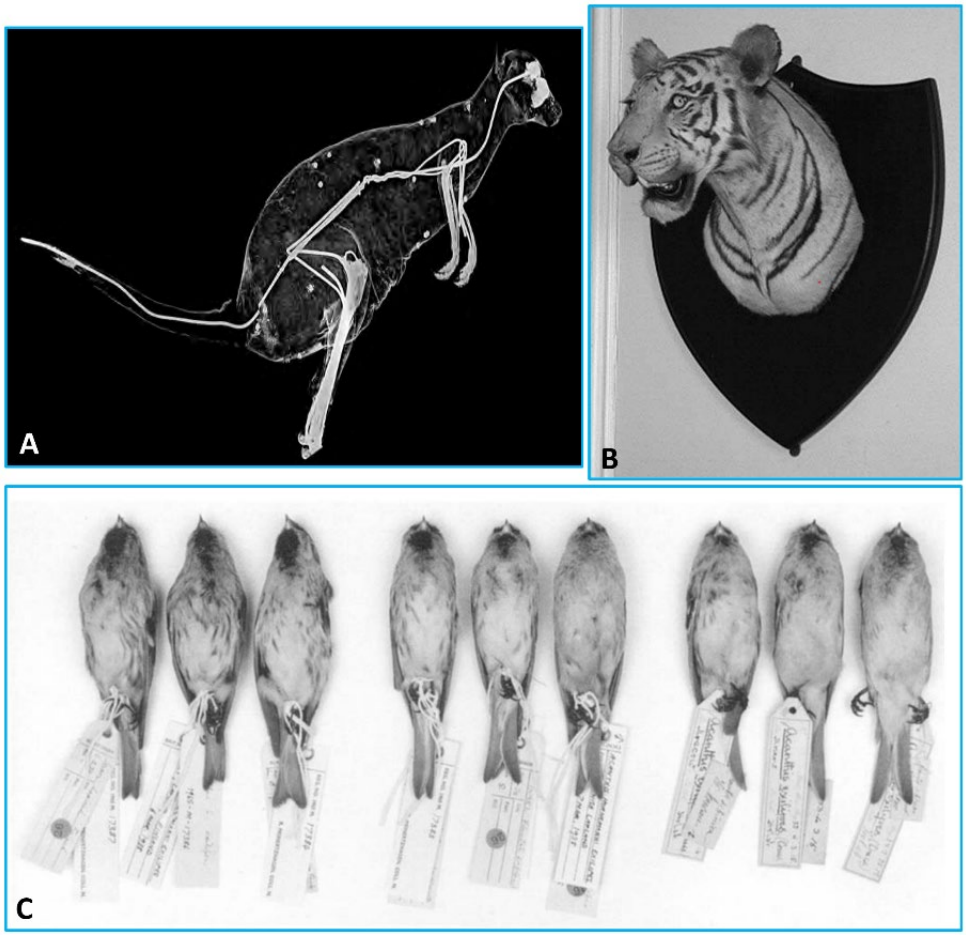

Fig. 1.2: Images that illustrate the different techniques by which stylistic characteristics can connect specimen with collector (**A**) X-ray radiographic image used to investigate internal structures (62) (**B**) Mounted tiger: the facial expression and shape of mounting boards were used for identification (65). (**C**) Visual comparison of specimens (66).

1.3.2 Chemical analysis

Chemical analytical methods have enabled specimens to act as an "historical witness" (50 p.250) to their histories. Yet as with all analysis, the outcome is dependent on the question asked of the data. DNA and RNA analyses have reinstated and renewed specimen identification data based on taxonomic frameworks at the time of examination (68-70). Isotopic element studies have been widely used in ecological and biological research to investigate the life of animal-specimens and successfully re-establish data such as geographic location and dietary

habits (71-73). This method, however, is rarely helpful in revealing missing information about the date of field collection, which has left many historic specimens eliminated from temporal studies.

Other studies have applied compositional chemical analysis to examine the "afterlife" (55) of specimens as museum objects to reveal their histories from the animal's point of death. One of the earliest investigations, undertaken by Hall, *et al*., identified elements in unexpectedly high concentrations and connected these to the preservatives and pesticides applied by humans in the production and maintenance of museum specimens (74). Few investigations into preservatives and pesticides have since been undertaken with the purpose of reviving data about the humans connected to their production. Strekopytov, *et al*. (75), used inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), Pequignot (76) used X-ray fluorescence spectroscopy (XRF), and Gil Macarron, *et al*. (77) X-ray computer tomography (CT), and Fourier transform infrared (FTIR) spectroscopy to investigate the methods of specimen production.

To date, the majority of chemical analyses have focussed on identifying and measuring toxic preservatives and pesticides because of associated health risks to collection users and exhibition audiences. These studies include work by Cross and Odegaard (78), Sirios and Sansoucy (79), Strekopytov, *et al*. (80), Bacon, *et al.* (81), Muir ,*et al.* (82), and Hamann (83). These studies have contributed to treatment histories of collections and individual specimens, but their limited focus has constrained their application in reconstructing the core specimen data suitable for other research endeavours. Yet, it is through these studies that XRF has emerged as the preferred analytical method for application in museum zoological collections.

1.3.3 'Non-destructive' chemical analysis

The biggest limitation faced by chemical analysis in the context of museum specimen studies is the requirement to minimise any loss of the original material to meet the museum's remit of preserving the collection in-perpetuity. Here the divergent terminologies used in chemistry and in museums complicate the issue (84).

In chemistry, the term 'non-destructive' is applied to analytical techniques that may require extraction of physical samples but do not destroy that sample. In the cultural heritage sector, the extraction of a physical sample from a specimen is a destructive intervention. Repeated sampling progressively diminishes the materiality of a zoological specimen as both a cultural object and a scientific resource, which limits future opportunities for research and education (85). Even the risk of damage to museum objects during transport to a laboratory for analysis is undesirable (86,87). Scientific communities are increasingly aware of the disadvantages of sampling museum objects (88,89).

This is precisely why XRF spectroscopy and particularly portable XRF instrumentation has become such a dominant technique for cultural heritage material analysis (90,91). Its use has gained wide acceptance in museums with many institutions routinely applying XRF for materials identification (92). This mutual understanding between scientists and museum staff aids in the timely progress of requests for permission to conduct XRF analysis (26).

1.4 Preserving historic skins for museum collections

1.4.1 Skins, spirits, furs, and mounted specimens

In a natural history collection, skin specimens typically take four forms. Each serve a particular purpose and are prepared and preserved in different ways as described below.

Mounted skin specimens: This is the skin specimen form typically associated with museum displays (93). A form beneath the skin enables the specimen to appear similar to the animal in life and this life-like appearance is augmented by artificial eyes. This form distinguishes mounted skins from other skin specimens. Their internal structures were made from a range of materials including plaster, clay, wood, rope, cotton, wool, plant material, and fibreglass (50,94). In the eighteenth and early nineteenth century, this was the dominant manner of preparing museum skins (10,95) used for display and for the identification and description of new species (50). As the enthusiasm for natural history became fashionable, mounted specimens appeared in homes and on hats (14).

Fig. 1.3: Mounted western barred bandicoot (*Perameles myosuros*) NHM463. A mounted skin specimen is characterised by a form that fills out the skin to replicate the shape of the living animal. Wires extending from the limbs enable them to be fixed to a stand. Image reproduced with permission from the Chau Chak Wing Museum, The University of Sydney.

Study skins: In these specimens there is little or no stuffing to support the skin and no attempt to replicate the eyes. The study skin emerged in the mid-nineteenth century in response to new methods of understanding natural history that required numerous examples of each specimen (96). Consequently, study skins are arranged to occupy minimal storage space.

Fig. 1.4: Study skin of Java mouse-deer (*Tragulus javanicus*) NHM174. Study skins are compact to save space, with little or no stuffing beneath the skin. Image produced with permission from the Chau Chak Wing Museum, The University of Sydney.

Hides and pelts: These are distinct from furs made into clothing and other items for consumer use. Natural history pelts are skin specimens cut open to lay flat (in two dimensions) and without paws, bones. Such collections are exclusively made up of mammals specimens.

Fig. 1.5: Pelts are typically flat with no remnant bones. Tasmania tiger (*Thylacinus cynocephalus*) specimen UMZC A6.7/3, University of Cambridge Natural History Museum. Image reproduced with permission © University of Cambridge / Jack Ashby.

Spirit specimens: Storage in 'spirits' enabled the preservation of the entire animal or its parts. These were collected for future research, or were reworked into a study skin or mount when the time and skills were available (11). In the eighteenth and nineteenth centuries liquids predominantly comprised of alcohol, often referred to as 'spirits of wine', were commonly (but not exclusively) used for this purpose (97). In the twentieth century, formalin mixtures came into use (97). A wide variety of specimens were stored in alcohol including minerals, reptiles, plants and mammals (98).

Fig. 1.6: Platypus (*Ornithorhynchus anatinus*) spirit specimen NHM1351. An entire specimen may be preserved in fluids. Image produced with permission from the Chau Chak Wing Museum, The University of Sydney.

1.4.2 The chemical nature of skin

Mammals have a skin structure comprised of three layers, the epidermis, dermis and hypodermis (99). The layers have similar molecular components, i.e., proteins,

polysaccharides, proteoglycans, cholesterol and lipids (99,100). However, the ratio and structure of these components varies between layers.

It is the presence of the proteins collagen, keratin and elastin, that make it possible to preserve skin (101). Of these, collagen is the most abundant by dry weight (101,102). Collagen molecules form a fascinating structure – a right-handed coil made up of three left-handed polypeptide helices twisted together (103). The structure is stabilised by hydrogen bonds and collagen fibres are connected by cross-linking covalent bonds (101,104). There are at least 28 different collagen types, seven of which bundle together to form fibrils held together by salt bridges (101,103,105). A schematic of these structures can be seen in Fig. 1.7A.

Skin decomposition begins almost immediately after death through the combined actions of microorganisms, enzymes and often insects (106). These processes may be hastened by high temperatures and relative humidity (106-108). The collagen structures, fundamental to the integrity of skin, denature mainly through oxidisation and acid hydrolysis (100,101,109). No longer able to hold a helical shape, collagen molecules ultimately become gelatinised and turn into a hydrated gel (101,105,109).

Preservatives prevent putrescence by inhibiting enzyme activity, making skin resistant to microorganisms, and deterring insect attack (1,110). Vegetable tans and metal salts act directly on collagen to replace intermolecular water and create cross-links within collagen structures that prevent denaturing (105,110,111) and contribute to microorganism resistance (112). Numerous metals, aside from the now popular chromium(III), can produce these tan-like interactions (113,114). Toxic metals, such as mercury (Hg), iron (Fe(II)), zinc (Zn) and lead (Pb(II)), can inhibit bacterial enzymes including those produced by Saprophytic bacteria, that live symbiotically in the fur and feathers of living animals (115,116). Oxidizable oils, including those from marine mammals and brain tanning, are water repellent and keep the skin too dry

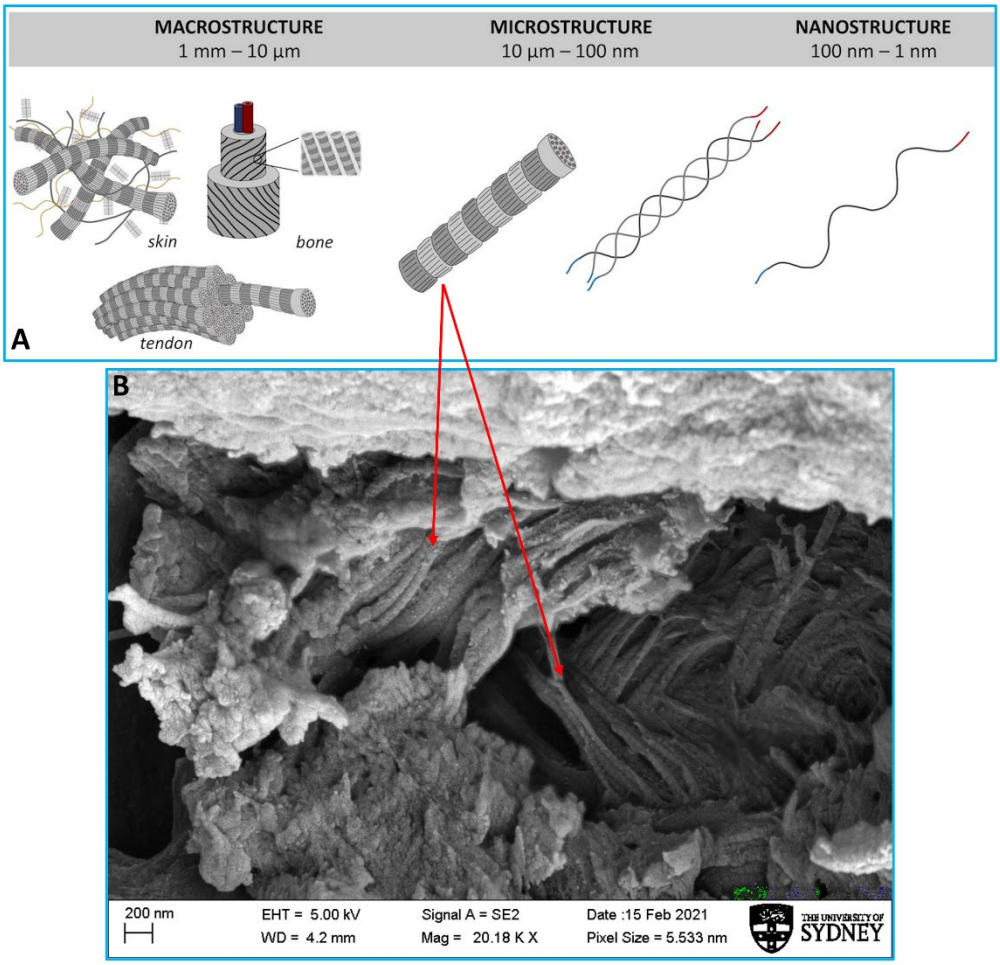

Fig. 1.7: Collagen fibrils in a museum specimen over 150 years old. (**A**) The organisation of collagen fibrils (103). (**B**) Scanning electron microscopic image of a sample removed from red winged parrot (*Aprosmictus erythropterus)* specimen NHM1354. Image produced with assistance from Sydney Microscopy and Microanalysis and with permission from the Chau Chak Wing Museum, The University of Sydney.

for bacterial colonisation (1). Fig. 1.7B presents a scanning electron microscopic (SEM) image collected from a sample removed from a red winged parrot (*Aprosmictus erythropterus)* specimen NHM1354, from the Macleay collection of the Chau Chack Wing Museum, The University of Sydney. This specimen, one missing its specimen data, was collected around the 1870s, and the intact collagen bundles demonstrate the efficacy of the preservatives used. Interestingly, even in the presence of toxic preservatives, historic museum specimens may be

inhabited by proteolytic bacteria (*Staphylococcus aureus* being the most abundant) and Aspergillus and Neurospora fungi (117,118).

1.4.3 Skin preservation in the nineteenth century

Field collectors of the nineteenth century rarely recorded the preservatives they used during expeditions (75,119). Our understanding of the preservatives and preservation techniques of the period is based primarily on published instruction manuals and pamphlets (50). Over 250 of these manuals were published between 1650 and 1950, each presented preservation methods, and preservative recipe, methods for their preparation, and instructions for their application (52). The number of skinning and preparation techniques for mammal skins were limited, and the stages in preserving and preparing a mammal specimen are presented in Fig 1.8. Conversely, the mixtures and ingredients of preservative recipes were numerous and diverse. Would writes that the ingredients of these recipes were an "odd assortment or preservatives and pesticides" (8 p.141).

A review of contemporary manuals (21,120-139) shows that field collectors of the nineteenth century chose preservation methods and preservatives after assessing a number of factors including;

- the type and size of the animal;
- rarity of the animal;
- thickness of the skin and seasonal variation in the fur/feather mass;
- weather conditions;
- available preservatives and tools and working space;
- practitioner's training or experience;
- period before shipping to the purchasing collector or museum; and
- transport method from the field.

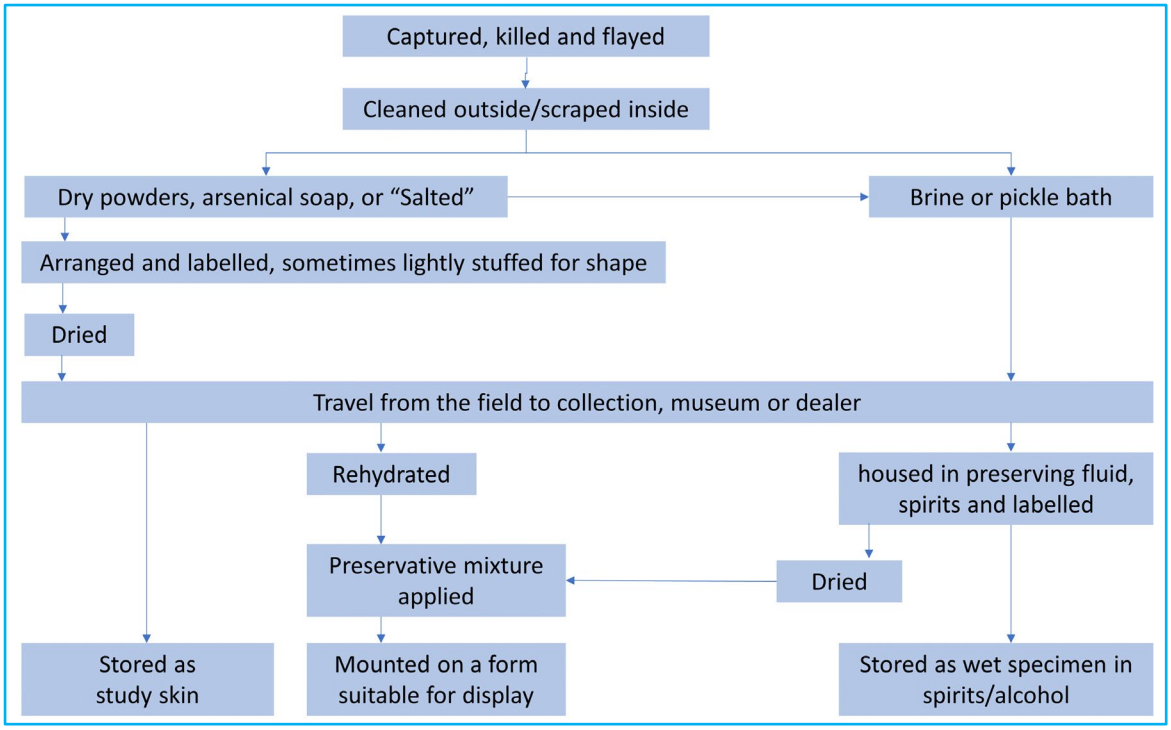

Fig. 1.8: Stages in preserving and preparing a museum mammal specimen.

In 1800, Nicolas noted that the preservatives recommended by his contemporaries fell into three groups – pungent herbs and spices, resins and oils like those used by the ancient Egyptians for embalming, and metal salts including those of copper, vitriol (iron) and alum (6). During subsequent decades, the availability of ingredients changed which resulted in the publication of recipes that relied more heavily on mineral salts and required fewer plant based materials (1,6). The simultaneous booms in leather production and ship building consumed tanning barks, such as oak bark, but industrialisation provided manufactured alternatives to naturally occurring minerals in abundance (1).

The recipe for arsenical soap was published shortly after Nicolas made his comments (64,140). While the invention of arsenical soap has been attributed to Jean-Baptiste Bécoeur (1718-1777) (140), at least one other French scientist was touting the benefits of suspending arsenic in a medium of soap. Phillipe Pinel, offered a recipe in 1791 for mammal skins that combined arsenic, brandy, aloe and "savon noir" (black soap) to be applied to the inner surface (flesh

side) of a fresh skin (125). These soap mixtures were proposed as a one-step preservative that could be premixed and put into action when the need arose. The use of arsenical soap removed one of the more cumbersome steps used in skin preservation – soaking the skin in barrels of brine before being dried and shipped to collectors and museums (125).

Twentieth century discussions about the history of natural history situate the invention of arsenical soap as a revolution in skin preservation that resulted in it being the most used preservative by the late 1800s (13,141,142). Based on the prolific republication of Béceour's original recipe, and variations, Farber asserts it became the standard preservative mixture by 1830 (143). However, few studies have investigated the uptake of this technology amongst collectors in the field, almost certainly because of a lack of primary evidence (documentary or physical) that showed which preservatives were used by individual collectors.

This research has investigated a small number of the preservative chemicals documented in the published instruction manuals of the eighteenth and nineteenth century. These are briefly discussed below.

1.5 Preservatives of interest

In this research, a number of compounds were recognised as interesting as representatives of technological changes in the preservation of natural history specimens. Two sources have catalogued many of the ingredients found in these recipes (6,52). However, changes in terminology over the centuries have complicated the identification and chemical composition of ingredients and this is made more complex by the simplified descriptions used by Williams and Hawks (52,144). What follows is a brief introduction to preservatives selected for enquiry in the research contained within this thesis.

1.5.1 Green vitriol

Among the chemicals called ‘vitriols’, green vitriol specifically referred to the iron salt, iron(II) sulfate ($FeSO_4$) and the naturally occurring mineral melanterite (144,145). Its earliest appearance in manuals for the preservation of natural history specimens is the instructions of Wolfgang von Hohberg (1612-1688) (146). Iron interacts with collagen to inhibit its degradation (113) and Rifkin has shown that it inhibits bacteria that produce the enzyme collagenase and the action of the enzyme itself (147). Iron salts have found use for darkening leather (148), as a mordant (149), and as a tanning or preserving agent (147,150-152). Depending on the pH of the skin, sulfur dioxide may become available and function as an antimicrobial (153).

1.5.2 Alum

In the eighteenth and nineteenth century, the term ‘alum’ was used for a number of different aluminium salts, including aluminium sulfate or paper makers alum ($Al_2(SO_4)_3$), and potassium aluminium sulfate ($KAl(SO_4)_2$). It’s earliest use as a skin preservative is found in Egyptian texts (115,149) and is one of the earliest preservatives described for use in the preservation of natural history specimens (143,154). The aluminium in this preservative bonds directly with collagen to form a moisture sensitive leather (114).

Related to other alums is ‘burnt alum’, ammonium aluminium sulfate ($(NH_4)Al(SO_4)_2$). Browne is careful to discriminate the two, advising that burnt alum is less likely to produce efflorescence on the skin when mixed with ‘carbolic’ and applied as a surface treatment to small areas such as the nose and tongue (120 p.231).

1.5.3 White arsenic

Of all the preservatives used in zoological specimens, arsenic (As) is the most commonly reported (52,155). The historic terms ‘white arsenic’, ‘arsenous acid’, or ‘white alum’ referred

to a powdered arsenic salt, most likely As_2O_3 (145). To date, the earliest documented use of arsenic for the preservation of zoological specimens was by Hohberg in 1682 (146). However, As was also used, in various mixtures, as a pesticide in museum to suppress insect and rodent activity (156). Recent research suggests that As may also function to stabilise collagen and keratin structures. As(III) salts are reported to interact with sulfhydryl groups present in collagen and keratin to crosslink protein structures (157,158). However, Hanacziwskyj, *et al,.* found that the application of arsenical soap did not improve the longevity of artificially aged skin samples (159).

1.5.4 Potash

More than one chemical was named 'potash' in the eighteenth and nineteenth centuries, all were salts of potassium, including potassium sulfate (K_2SO_4), potassium aluminium sulfate ($KAl(SO_4)_2$), potassium nitrate (KNO_3), and potassium chloride (KCl). In Europe, the use of potash in preserving skins can be traced to the mixtures used by medieval skin and fur workers (whittawyers, curriers and skinners) when wood ash was used as the source of potassium (1,6). KCl has recently been reported to be as effective as a preservative as sodium chloride (common salt) in high concentrations, due to its ability to supress microbial activity by extracting water from the skin (153).

1.5.5 Saltpetre

Saltpetre historically referred to potassium nitrate (KNO_3) and was also known as 'nitre' and 'potash'(160,161). Medieval Europeans used it as a preservative for meats (162), and from as early as 1555, it was applied in the preservation of natural history specimens (143,154). Its use in the preservation of Australian specimens was recorded in a letter from the Australian Museums first field collector, William Holmes, who requested supplies for preserving birds in 1829 (163). It is the nitrate in saltpetre which, once reduced to nitrite, reacts with lipids to

inhibit oxidisation, and the activity of bacteria, especially *Clostridium botulinum* (153,161,164). Saltpetre is used as a food preservative today and is referred to as E252 on food labels.

1.5.6 Mercury chloride

Mercury chloride ($HgCl_2$) is commonly found in museum zoological collections (165). Its earliest use for the preservation of zoological skins was in 1696 (166). In the nineteenth century $HgCl_2$ was known in as 'calomel', 'mercurous chloride' and 'corrosive sublimate'(167), and was applied to both inner and outer surfaces of a zoological skin (168). Even as late as the 1940s, $HgCl_2$ was considered to be only temporarily effective as a pesticide and frequent reapplication was recommended (75).

Hg binds to sulfhydryl, amide and amine groups within proteins (52,169,170). The literature also suggests that $HgCl_2$ decomposes into elemental Hg when exposed to organic matter in light (171,172), which them sublimes to a gas potentially acting as a fumigant (51). $HgCl_2$ is still used today as a fixative in B-5, Helly's and Zenker's solutions.

1.5.7 Zinc salts

Named 'white vitriol' (145), zinc salts are rarely mentioned in the historic manuals for preservation of zoological specimens. Only zinc chloride ($ZnCl_2$) has been connected with historic zoological collections, and always in associated with wet specimens (21,50,120,173-176). However, zinc sulfate ($ZnSO_4$) is currently used as a fixative in the preservation of zoological specimens (177,178).

1.5.8 Lead salts

Lead was used in the form of the pesticide lead arsenate after 1892 (156). Although lead salts are not referred to in historic manuals, lead was found in significant concentrations, and uncorrelated with As concentrations, in historic specimens at the Horniman Museum, London,

and at the Muséum National d'Histoire Haturelle, Paris. At the Horniman, Bacon *et al.,* attributed the presence of lead to a possible application of petrol as a pesticide (81). Pequignot attributed lead to dust from lead paint used on the walls at the Muséum National d'Histoire Naturelle (179). Strekopytov, *et al*., also found significant concentrations of lead in samples removed from historic bird specimens, and attributed it to lead shot likely used in field collection (75).

1.5.9 Ammonium chloride

Historically named sal ammoniac (NH_4Cl), this preservative appears in a small number of recipes including Browne's bath made by mixing $HgCl_2$ and 'carbolic', and used to halt an insect infestation (120). It was also recommended by Ellis, mixed with $HgCl_2$ to prevent pest damage to botanical specimens in transit (166).

1.6 Knowledge gaps

The survey of literature on nineteenth century preservatives identified a number of knowledge gaps.

There are few published studies that investigate the extent of missing specimen data in museum collections, and none that focus on data missing from the oldest and most rare specimens in collection. This is surprising considering the literature acknowledges that data loss has a considerable impact on environmental, ecological and biological studies (20,28,29,32,44,180).

There is substantial literature on the evolution of preservation practices during the Enlightenment. However, the preparations used by individual naturalists are "largely unrecognised" (181 p.163). The literature has repeatedly acknowledged a lack of records which link preservative and pesticide treatments with specific specimens (74,168,182-185). While some studies have attempted to re-create this history of specimens, very few have utilised non-

destructive (non-invasive) techniques. Of those techniques applied, nearly all require a great deal of effort to produce results for only a few specimens.

The literature that discusses the history and development of preservation and taxidermy places much importance on the invention of arsenical soap. There are no published investigations that demonstrate how rapidly arsenical soap was adopted by museum field collectors and taxidermists, nor when the invention was adopted in far-flung collecting locations and colonial territories.

In investigating the protocols previously used for applying portable XRF spectroscopy to zoological specimens, it was clear that very few publications include the experimental parameters required to reproduce the results reported. Deering, *et al,*. provided only the voltage setting (186). Pequignot provided measurement time and the number of sites measured on each specimen, but no further details (76). Only the authors Shugar and Sirios discussed the complete experimental details in their publications (168,187). Several authors reported quantitative results, in parts per million, but do not specify the calibration (methods) used nor the release date of commercial methods that might enable further investigation of the instrumental parameters preset within many instrumental calibrations (186,188-190).

1.7 Overarching research aims

This research project aims to develop a protocol to re-connect ‘no data’ specimens with their contextual specimen data. The museum specimen is framed as a cultural object. The conceptual connections, made in previous specimen studies, between the materiality of a specimen and its maker are developed. The protocol brings the assertions made by Faber and Pequignot that innovations in skin preservation may be representative of the time of manufacture (50,143), together with Prŷs-Jones’ observation that individual naturalists used different amounts of preservative (61). This investigation is founded on the notion, presented by Patchett (191) and

van Allen (47), that a craftsperson or practitioner will repeatedly use similar materials and techniques in their work. These may be used as identifying markers.

In the research described in this dissertation, the chemical composition of preservatives, applied to the animal-specimen at the point of capture in the field, are investigated to determine if these are characteristic of the craft practice of individual field collectors. By identifying the field collector, it is postulated that a specimen may be reconnected to the documentary records of its collection. A passage for finding missing specimen data within the documentary evidence may open, not back in time from the present museum specimen to its past, but forward in time, from field-collection to the present.

The specific research aims are as follows;

- establish a reproducible and object-safe protocol for rapid collection of compositional data from zoological specimens that is both affordable for museums, and can be executed by museum staff without significant additional training;
- establish a robust XRF data collection and analysis protocol that enables comparison between specimens, and may facilitate the identification of the treatment histories for groups (rather than case-by-case);
- test the hypothesis that preservative residues can connect individual specimens with their field-collector or maker;
- connect the literature about the history of preservation together with physical evidence to contribute to the history and evolution of specimen preservative use in Australia; and
- reclaim the field collection history of at least one ‘no data’ zoological specimen.

1.8 Who will benefit from this research?

Natural History Museums are faced with competing priorities – to facilitate the use of zoological specimens as research resources, while simultaneously preserving specimens from damage so that they can remain a resource. This is undertaken within the context of diminished funding and continually expanding collections (192,193). The outcomes of this research respond to this paradox.

By reconnecting 'no data' specimens with their histories and re-establishing core specimen data, these specimens can be included in biogeographical and ecological research. This has benefits for researchers as increased biological resources may lead to broader, more impactful observations, and more robust studies undertaken with larger numbers of replicates (26). Where older specimens are returned to the resource pool, the time frame of studies may be extended (32). For museums, increasing the functionality of the specimen collection for research justifies the expense of its housing.

The preservatives and pesticides used on specimens impacts their use in further chemical studies e.g., genome or pollution studies (26,74,194,195). By better understanding the preservatives used on each specimen, any necessary destructive (physical) sampling can be directed towards those specimens most likely to yield successful results. The development of non-destructive screening protocol would preserve unsuitable specimens from destructive sampling.

Improved knowledge of specimen preservatives has other positive outcomes. Efforts to preserve zoological specimens from decay and damage are hampered by the complicated mixture of chemicals used in their preservation (159). Some conservation treatments have the potential to worsen rather than remediate specimen condition. A better understanding about the

preservatives used in each specimen, therefore, can inform materials conservation decision-making.

Finally, the cost of chemical analysis can be significant enough to eliminate investigations of large groups of specimens. A portable XRF instrument is relatively cost effective to purchase but more critically, both the instruments and the skills to use them already exist in many museums. A protocol that utilises existing resources, or those that can be accessed within a tight budget, makes the survey of large groups possible.

1.9 The structure of this dissertation

This dissertation has been written in a manuscript format. Each progressive chapter documents a discrete stage in the passage from concept and development of a protocol, through proof-of-concept testing, to the demonstration of successful outcomes.

In *Chapter 1*, the problem of lost museum specimen data is introduced. The aim of the research is discussed and a background to understanding skin and its preservatives is presented. Portable XRF spectroscopy is identified as the preferred analytical method.

Chapter 2 presents the complexities of using portable XRF spectroscopy for investigating skin samples and describes the development of the XRF protocol used throughout this dissertation. It concludes with the recognition that chemometric analysis is necessary for exploring the historical use of preservatives and pesticides in museum zoological specimens.

Chapter 3 explores the nature of historic specimen data and the mechanisms of data loss in the context of natural history collections. Discussed here is the importance of understanding the character of museum specimen data before selecting the specimens that form a ‘known’ reference set from a comparative investigation. It concludes with detailing the criteria used in selecting specimens for the research described in this thesis.

Chapter 4 documents the application of the XRF protocol in combination with principal component analysis (PCA) for interpreting the residues of preservative treatments. This chapter demonstrates the potential of combining portable XRF and PCA for investigating the relationship between chemical composition and field collector.

Chapter 5 describes the application of the developed portable XRF/PCA protocol to discover that high concentrations of zinc in dry museum specimens are characteristic of historical storage in ethanol. The XRF/PCA protocol is presented as a potential screening method to discriminate among specimens for further chemical analysis, including DNA studies.

Chapter 6 describes the XRF/PCA protocol used to determine the elemental characteristics that discriminate among specimens prepared by different field collectors. These characteristics were used to connect 'no data' specimens from the Australian Museum (Sydney) with the prolific field collector, George Masters. Through this connection, 'no data' specimens were reconnected to the documentary records of their field collection and their specimen data were re-established.

Chapter 7 summaries the conclusions of the research and future directions.

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Chapter 2

Recognising Remnant Residues

2.1 Introduction

Museum specimens are, by their very nature, extraordinarily diverse (1). Yet, comparison between specimens is essential if the craft practice of field collection and preservation is to be assessed. To facilitate comparison between specimens, this Chapter discusses the sources of heterogeneity within and between specimens and summarises the well-documented theory of X-ray fluorescence (XRF) spectroscopy in the context of zoological museum specimens (2-4). Together, those sources of heterogeneity and the nature of XRF have informed the design of an experimental protocol which included data collection and correction, and the conceptual principles behind the data interpretation.

While the X-ray fluorescence intensity observed during XRF elemental analysis is proportional to the concentration of elements within the sample, it is differentially modulated for individual elements by the interactions of both the incident X-rays and fluorescent X-rays generated during analysis by the sample matrix. The sample matrix refers to the physical structure of the sample and its elemental composition (5). Markowicz warns researchers to carefully consider the effect of the sample matrix when developing non-destructive and *in situ* experimental protocols (6). The application of pXRF spectroscopy to zoological museum specimens is complex, partly due to the sample matrix. The first section of this chapter outlines the matrix effects relevant to this research and presents the procedures and mitigations chosen to achieve good quality XRF data, accurate identification of the elements within the spectra, and corrections to the data to account for matrix effects.

In Section 2.4 of this Chapter, portable XRF (pXRF) is used to analyse a set of reference standards generated by treating fresh pig skin with a range of chemical preservatives

recommended in natural history manuals of the eighteenth to early twentieth centuries. Inductively coupled plasma mass spectrometry analysis (ICP-MS) was subsequently undertaken to obtain the quantitative elemental composition of each reference standard. The XRF spectra were deconvoluted and elemental analytical results were compared with the ICP-MS results to obtain a practical understanding of XRF interactions and instrumental sensitivity when analysing skin samples. The lessons learned using these reference standards were then applied to the deconvolution of XRF spectra collected from samples removed from two historic museum zoological specimens.

2.2 Heterogeneity, XRF spectroscopy and museum specimens

Skin is a complex and heterogenous tissue (7,8). Over the surface of a single animal, there are variations in skin density and in the thickness of the hair/feather layer (1). Further heterogeneity is introduced in the act of changing an animal into a zoological specimen. Preservatives were manually applied to skins, often using a brush (9,10), which produced variations in distribution and concentration over the animal surface (11), i.e., when the application brush is freshly loaded it will deposit a thick layer of preservative on contact with the skin surface. As XRF spectroscopic analysis penetrates beyond the specimen surface (12), the retention of bones within the specimen, and the use of internal structures and stuffing, may produce additional heterogeneity in the elemental data collected from a single specimen.

Once accepted into a collection, a specimen may be reworked, or sections repaired, which can introduce localised inconsistencies in elemental composition. Pesticides used in a museum and manually applied introduce further heterogeneity over the animal surface (11). For example, the broadcast of a pesticide powder onto study skins within a cabinet drawer may result in higher pesticide concentration on the upper surface of each specimen. Some specimens may have received multiple pesticide treatments since their acceptance into a museum collection

(13,14). The schematic in Fig. 2.1 illustrates how layers of manual treatments may result in irregular distribution of chemicals.

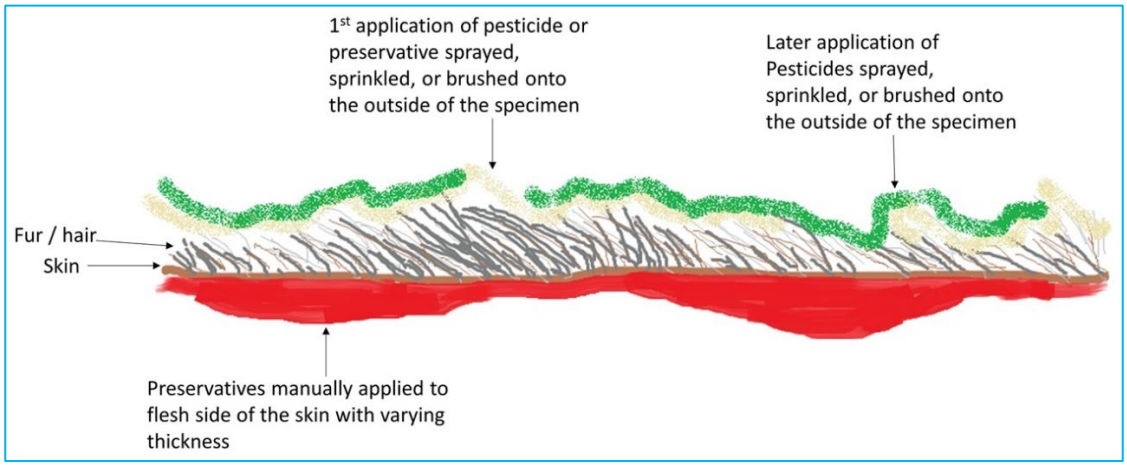

Fig. 2.1: Schematic diagram representing a specimen skin cross-section with preservative applied to the inner, flesh side (red), and pesticide applications to the outer surface (yellow, green).

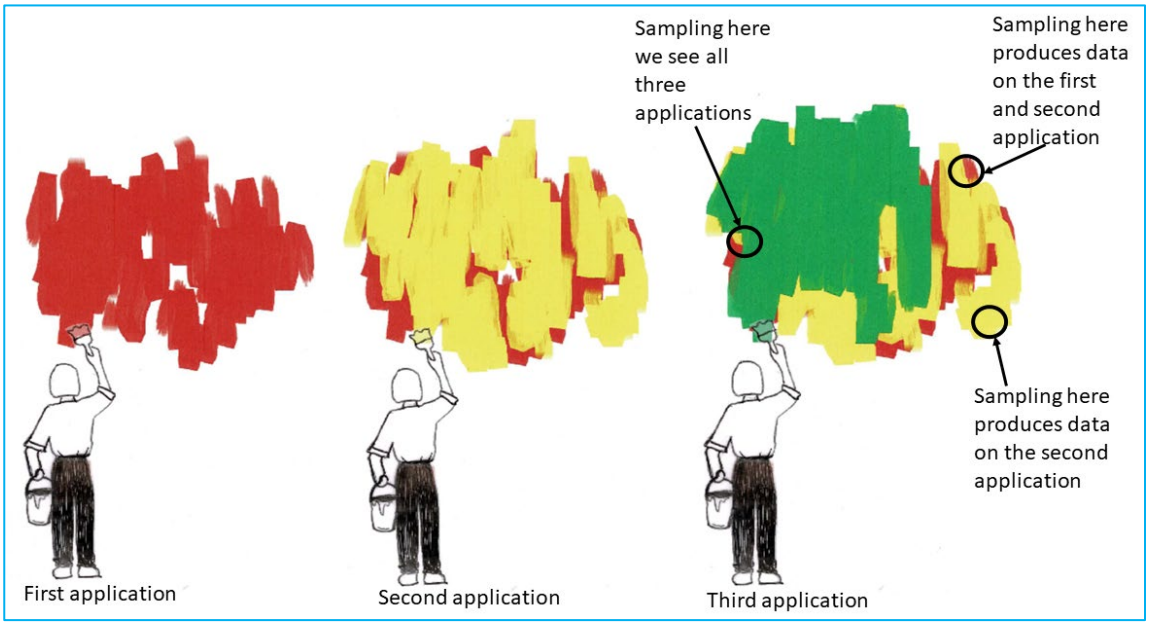

Fig. 2.2: Multiple layers of hand applied preservatives and pesticides are characterised by sites of thicker or thinner application resulting in heterogeneous distribution across the surface of a single skin. This can be likened to applying several coats of paint via brush.

When a single animal is analysed via XRF spectroscopy, disparity within a specimen can result in significantly different compositional data from one measurement site to another (15). An analogy of these preservative, repair, and pesticide layers is illustrated in Fig. 2.2, in which the painter applies multiple uneven layers producing different observations at various sampling sites. It can be extrapolated that such irregularities may be expected in observing individual museum specimens and as is evidenced by the photographs of zoological specimens presented in Fig. 2.3.

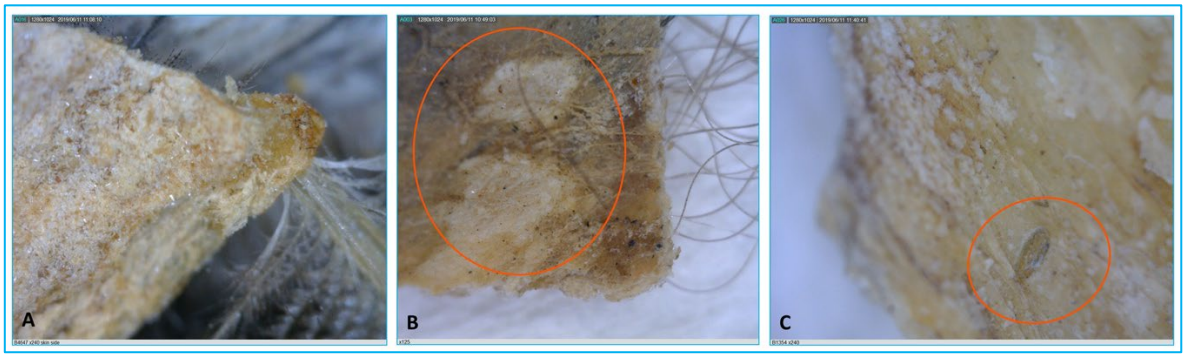

Fig. 2.3: Photographs of samples removed from three zoological museum specimens. (**A**) Flesh side of a preserved Kākāpō (*Strigops habroptilus*) skin NHB4647 showing a diversity in crystal sizes (magnification ×240). (**B**) Flesh side of a *rufous bettong (Aepyprymnus rufescens)* NHB457 showing variable distribution of preservatives including distinct white patches (magnification ×125). (**C**) *Red winged parrot (Aprosmictus erythropterus)* skin NHB1354 with a parasitic louse embedded in the preservative demonstrating that a single analytical measurement may capture the composition of more species than expected (magnification ×240).

Such variations become significant complications to correlative analysis when comparing one museum specimen to another. Dissimilitude between specimens may be influenced by age, sex and species (16,17), and endogenous chemicals accumulated during each animal's life via diet, geographic location, and habitat. The inclusion and type of internal structures, including the

retention of certain bones, were dictated by innovations in the profession, the preparator's preferences, and available resources (18,19). This creates heterogeneity when comparing specimens collected by different individuals or groups or prepared at different times over the nineteenth century. Additionally, different museums (and indeed storage locations) introduce exogenous contaminates to the specimen, including fine dusts and environmental contaminates, such as pollution from near-by roads and traffic, and pesticides applied within the museum to control pests (15). Each of these contaminants contain different chemicals. Specimens exchanged between museums are likely to have acquired contaminates from each location.

2.3 Data collection, comparability, and accounting for matrix effects

X-ray fluorescence is produced when an incident X-ray beam excites electrons of the elements present within a sample. With sufficient energy, an electron escapes its orbit creating a vacancy that is quickly filled by a higher-energy electron from another orbit or shell (20) (Fig. 2.4A). To make this transition, the replacement electron releases its excess energy with the emission of a fluorescent photon which has an energy that is characteristic of both its origin element and its orbit or shell (4) (Fig. 2.4B). The X-ray beam creates multiple events inducing shell vacancies, and corresponding fluorescent emissions, each characteristic of an element. An energy dispersive XRF spectrometer, such as the portable XRF spectrometers, simultaneously detects the characteristic photons produced by the sample, sorts them according to their energies, and ultimately generates a spectrum (21,22). The area under each peak that appears in the spectrum is proportional to the concentration of the elements within the sample (23), in thin samples that relationship is linear (24).

The most frequently occurring vacancies are created in the inner K shell orbital (called the 1s orbital in the quantum - mechanical model), producing K fluorescent lines in the XRF spectra.

However, vacancies may occur in the M (3s, 3p, 3d) and L (2s, 2p) orbitals creating M and L fluorescence lines. The annotation for fluorescence identifies both the location of the vacancy and the origin of the replacement electron. For example, Kα refers to a K shell vacancy that is filled with an electron from the L shell. In some cases, an element will reabsorb the energy emitted in the initial electron transition causing the ejection of a second electron. Ultimately, an unexpected photon is released (25). This is called the Auger effect.

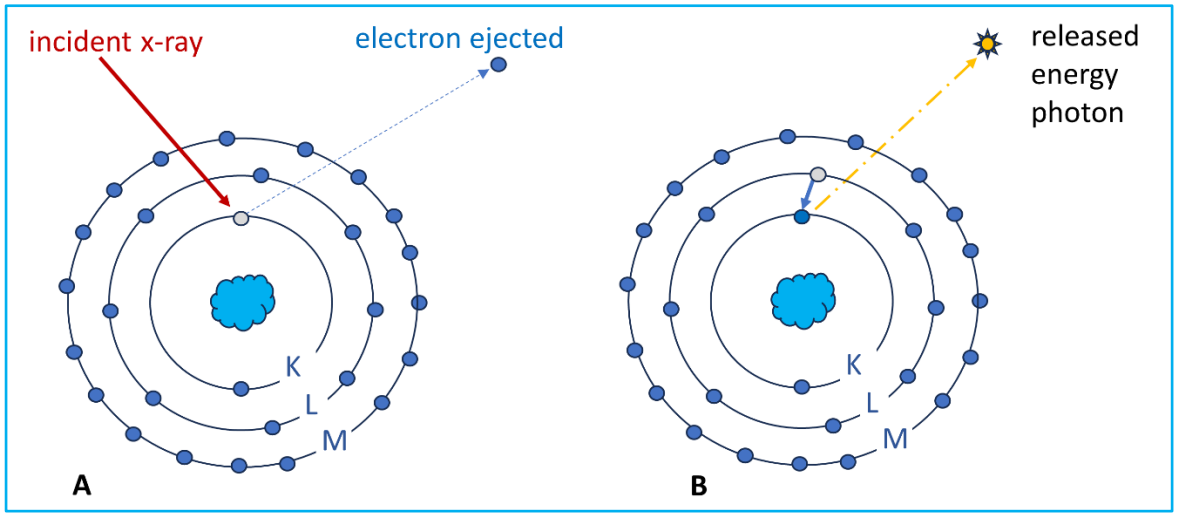

Fig. 2.4: Schematic diagram of the production of X-ray fluorescence using Bohr's atomic model. In **A**, an electron, excited by the incidence X-ray beam, is ejected from the inner electron shell. To maintain the stability of the atom, an outer shell electron must fall into the vacancy made in the inner shell. To do this, the electron releases excess energy, a fluorescence photon (**B**).

2.3.1 Sample density, thickness, and surface irregularity

Non-destructive XRF analysis (without physical sampling) of historic zoological specimens typically demands the collection of data from the outer surface. Fur and feathers form an irregular surface, and a low density layer that includes numerous airgaps. Shugar and Sirios demonstrated that thicker fur results in lower XRF counts (11). The skin beneath this layer will

differ in thicknesses and density especially when comparing animals of different species. This will yield variations in spectral peak intensity and width, and the appearance and location of spurious peaks (4,11). Low density matrices, including the skin and fur of museum mammal specimens, are associated with high scattering of the incident X-ray beam producing broad Compton scatter peaks (incoherent, inelastic scatter) (4,26) and Bremsstrahlung backscatter curves (11) (Fig.2.5). These may complicate the interpretation of low atomic (Z) weight and trace elements (4,27).

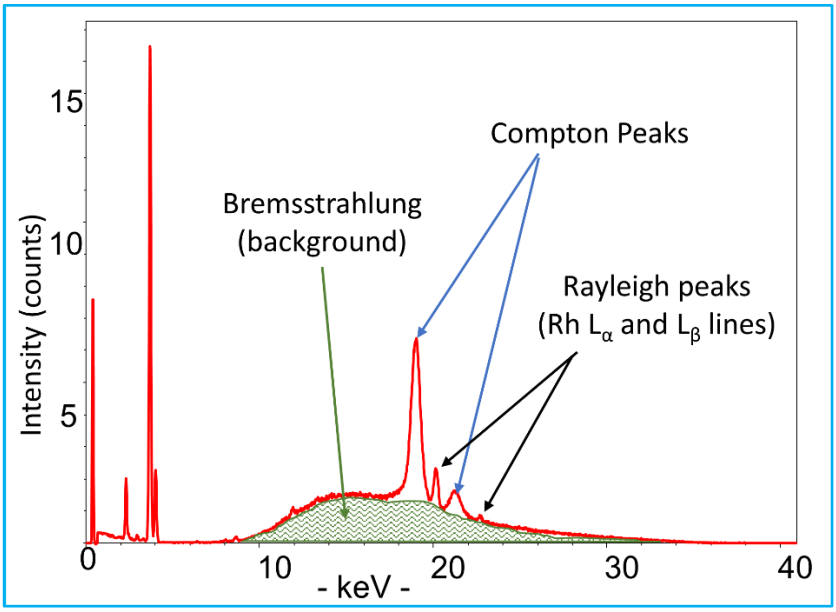

Fig. 2.5: Example of Bremsstrahlung continuum, Compton peaks, and Rayleigh peaks.

Additionally, surface irregularities and curvature can produce lower count rates due to the combined impact of air gaps and imperfect angles of incidence (6,28-30). The impact of these airgaps becomes significant when larger than 3 mm for high Z weight elements, but small gaps can affect the detection of elements with atomic numbers below sulfur (26,30,31). Convex surfaces, in particular, decrease the detection of photons produced by both light and heavy atomic weight elements (29).

To mitigate the influence of specimen shape, the fur layer, and matrix effects associated with sample density, *in situ* data collection was handled in the following ways:

Fig. 2.6: The custom built specimen stage allowed the specimen to rest, fully supported above the pXRF spectrometer.

1. Reduce inter-specimen variations. To eliminate the dissimilitude between fur and feathers, only mammal specimens were selected. The choice was made in part because of the large air gaps created by feathers, and because the natural growth patterns of feathers produce an uneven distribution of endogenous contaminates across the surface of single specimens (32), introducing an additional source of variation.
2. To reduce variation in both skin and fur thicknesses between specimens, the specimens were restricted to two genera of marsupials *Thylogale* spp. *(*family *Macropodidae)* and *Perameles myosuros (*family *Peramelidae)*. This action reduced the diversity of endogenous elements caused by significantly different diets (e.g., omnivorous vs herbivorous diets).

3. The selected specimens were predominantly study skins which are ‘flatter’ in shape than mounted specimens. This enabled relatively flat surfaces to be presented to the nose of the instrument. In selecting sites for XRF measurement, concave areas of each animal were avoided.
4. A custom built specimen stage was created that enabled the specimen to rest above the nose of the instrument, and the fur to be compressed only under the weight of the specimen itself. This reduced the air spaces with the added benefit of limiting the risk of excessive compression and associated damage to the fur (Fig. 2.6). The stage was constructed from corrugated polyethylene board 450mm × 300mm with a void in the centre to accommodate the nose of the pXRF. Legs of the same board type were attached with stainless steel brackets and fixings. All corners were rounded to prevent accidental damage.
5. A filter (25 μm Ti/300 μm Al) was fitted to the instrument to mitigate the Bremsstrahlung continuum curve and improve identification of trace elemental peaks (11).
6. Data were collected from numerous sites across a single skin to represent the entire specimen, which included the different fur and skin densities at each measurement site (33). In this respect, the older specimens of *Thylogale* spp. were ideal, as each specimen had areas of very little fur on the under-side of their hind legs allowing measurement of the skin alone (Fig, 2.7 below).

2.3.2 Data analysis approach to account for sample matrix effects

Appropriate data analysis offered further opportunities to account for density, thickness, and surface irregularity. Commercially available instrument calibrations (sometimes called methods or modes) are typically used to account for variations between inorganic materials,

and simultaneously provide quantitative results in a percentage or parts per million (34). However, there is currently no available calibration for an organic matrix (35). Several studies

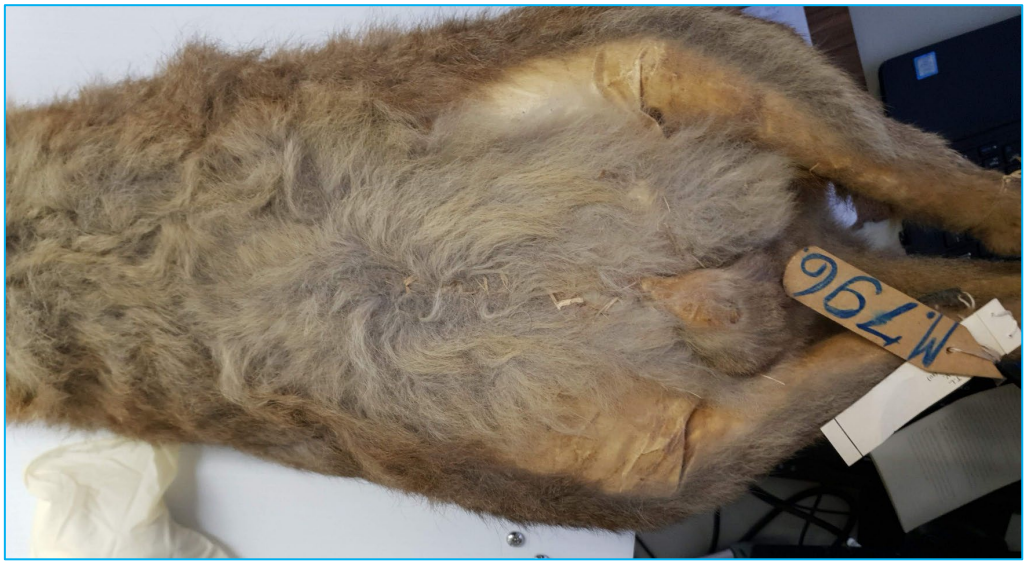

Fig. 2.7: Older *Thylogale* spp. specimens had very little fur on the under-side of their hind legs which enabled measurement of the skin alone.

have shown that existing commercial calibrations do not provide reliable results for zoological and ethnographic objects with high biological composition (36-38). This is attributed to the significant difference between the matrices in the material of interest and those of the calibration standards (6), and erroneous algorithmic peak identifications for overlapping peaks including arsenic and lead (31,39). This issue is exacerbated by a 'closed sum effect' in which the estimated percentage of light elements (undetectable using pXRF) in the standards are significantly lower than the proportion of these elements in zoological specimens (40-42).

Custom calibrations combined with fundamental parameter computations offer a solution by enabling the researcher to create case-specific calibration standards that better reflect the matrix of zoological specimens (43). However, custom calibrations must be undertaken for each instrument using the same calibration standards. When the standards include toxic elements,

such as arsenic and mercury, the handling and transporting of a single set of calibration standards between locations (in this case museums) may be legislatively constrained and was considered undesirable.

The remarks of Löwemark, *et al*., on the benefits of XRF analysis for comparative studies (41), led to the recognition that absolute concentrations were not necessary for this study - the relative elemental concentrations were sufficient for discrimination between specimens. Shugar points out that clear groupings can be identified without quantitative calibrations by observing the ratios of compositional elements with the condition that the samples have similar matrices (23 p.190). Because available calibrations may produce false absolute concentration values, the recommended use of semi-quantitative net peak area values to limit sources of error (29) was adopted for the research described herein.

While the data collection protocols, described above, ensured some similarity among samples, the connection between the sample density and Compton scatter peak area presented an opportunity to account for density and thickness variations and mitigate the impact of air gaps and surface irregularities (5,26,44). Hence, each spectrum was normalised to its own Compton scatter peak area. Processing the data in this manner enabled the protocol to be applied without the expense of commercial calibrations or the risks associated with toxic calibration standards. However, this correction is not as effective for low Z weight elements (5). As such the counts reported for these elements were treated with a degree of caution (29).

For successful comparison among specimens, the same instrument configuration must be used to collect data for each to avoid variation in scatter angle, differences in radiation source and intensity, and variation in instrumental noise (5). Furthermore, data collection was carried out at approximately the same time in the life of the radiation source (28,41).

2.3.3 Sample penetration

Both the depth at which elements can be detected using XRF spectroscopy and the capacity for the fluorescence photons to escape the sample is determined by the nature of the matrix and by energy of the escaping fluorescence. Thus, photons absorbed and produced by low Z weight elements are most affected by the sample matrix due to their lower energy (23,31). Potts estimates that low atomic weight elements may be detectable in the micrometre range while "higher atomic elements" may be detected to a 10 mm depth (28 p.5). This presents two complexities to be overcome when investigating historic zoological specimens, the influence of surface pesticides, and the impact of internal structures.

The first consideration is that elements present on the specimen surface are more readily detected than those below the surface (6,39). This enhances the contribution of surface treatments to the data collected, e.g., pesticides applied to specimens while in a museum collection. This was addressed by the implementation of the following protocols.

1. Measurements were collected from numerous discrete sites on each specimen, to allow for the interpretation of the distribution pattern of preservative elements, i.e., even, patchy, or localised (45). Even distribution of treatments applied to the fur side of a whole specimen (either study skin or mounted) would be difficult to achieve, especially in a museum scenario where many specimens would be treated at the same time via band spraying or fumigation. Conversely, treatments to the flesh side of the skin, when it was laid out flat, were likely to be more evenly distributed. Thus, elements with high variability in distribution over a single specimen may be interpreted as indicators of a treatment to the outer surface especially if the specimens skin thickness is relatively uniform.

2. To limit the variability of contaminates resulting from different museum environments and pesticide programs, specimens were selected from two museums: the Australian Museum, Sydney and the Macleay Collection in the Chau Chak Wing Museum (The University of Sydney). The museums are located within close proximity of each other, and their collections were established at a similar time using a similar network of specimen suppliers.

The second consideration is that X-rays may penetrate beyond the fur and skin layer into the internal stuffing and structures, producing data that contains elemental information unrelated to the skin and its treatments. An X-ray radiograph was collected from a sample specimen, M128, to investigate the structures used to support the physical shape of a *Thylogale* sp. skin (Fig. 2.8). Using the X-ray radiographic image as a guide. measurements were taken from areas of M128, and all subsequent *Thylogale* spp. specimens, where wires and bones were unlikely to be close to the surface, e.g., as such the tail and lower portion of the hind limbs were avoided.

2.3.4 Mineralogical effects

Many nineteenth century preservatives contain crystalline material, e.g., potassium aluminium sulfate or alum. Markowicz cautions that any single measurement may be influenced by the size of particles within the sample. Both the incident X-ray beam and subsequent fluorescence may be absorbed by large particles or reflected from crystalline surfaces (6,31,46). Crystalline surfaces can produce Bragg scatter and offset spectral lines (emissions created by the phenomena of X-ray diffraction) and these are more readily observed in samples containing low molecular weight elements (5,31). Amar demonstrated that larger crystals produced a greater error and attributed some of this error to the airgaps between particles causing attenuation (47). The impact of mineralogical matrix effects was mitigated by fitting the pXRF spectrometer with an 8 mm collimator to produce the largest possible spot size and to reduce

the likelihood of a single crystal dominating the data. Multiple measurements from discrete and non-overlapping sites from each specimen and the removal of significant outliers in the data were also used to mitigate the impact of mineralogical effects (26,29).

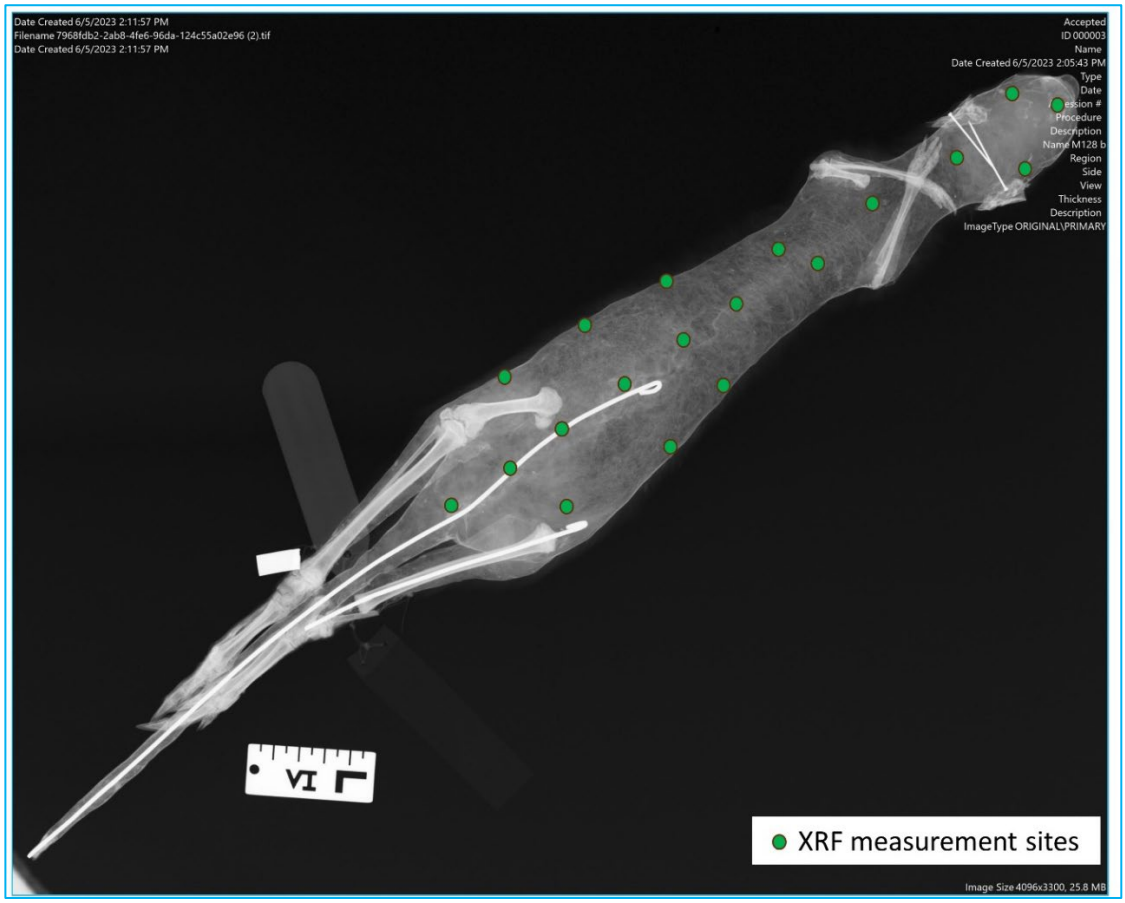

Fig. 2.8: X-radiograph of specimen M.128 showed the typical substructure of a *Thylogale* study skin. This information was used to guide the selection of appropriate measurement sites, marked in green. Radiograph produced by the Australian Museum using Thermo Fisher Scientific industrial X-ray, Model PXS 5-927EA at 40 kV and 180 µA, 7 s exposure.

2.3.5 Chemical matrix effects

Certain elements will enhance or absorb the fluorescence created by other elements, as characteristic X-ray photons emitted from one element may excite other elements in a phenomena called secondary and tertiary fluorescence (39,48). The preservatives and

pesticides applied to historic zoological specimens include elements known to create chemical matrix effects. For example, the presence of calcium present in the arsenical soap ingredient, rock lime, may enhance the detection of mercury, lead, and potassium (12), while elements such as lead, will restrict the escape of fluorescent photons (12). Table 2.1 (below) lists the matrix effects that impact the investigation of museum mammal specimens using XRF spectroscopy. This information was used throughout the following Chapters in the interpretation of XRF data.

2.3.6 Moisture effects

Moisture can absorb X-rays and increase the scatter of fluorescence thus diminishing the peak intensity of the elements detected (6,30,47). This presents some interesting considerations when analysing zoological museum specimens. While museums typically take great care to control relative humidity surrounding their collections, unbound water absorbed into skin due to locally high relative humidity may present a problem when comparing specimens from storage locations with different relative humidities. However, both Potts et al., and Markowicz reported that normalising XRF data to the total area of the Compton peak (incoherent scatter) can correct for moisture effects (5,26).

Water bound within the molecular structures is reported to have little impact on XRF analysis of geological samples (49) however, there is no published literature that investigates the effect of intermolecular water loss on the detection of elements in zoological material via XRF spectroscopy. The molecular structure of collagen alters as skin ages and the quantity of intermolecular and intramolecular water may change (50). This potentially impacts the peak intensity of analytes in zoological specimens that have greater collagen deterioration (due to age or damage) when compared to specimens with more intact collagen structures.

Consideration was given to this possibility in interpreting relative elemental concentrations when comparing specimens of significantly different ages.

Table 2.1: Common chemical matrix effects relevant to the study of zoological specimens.

Element	Interactions
Calcium (Ca)	• Ca enhances the detection of sulfur (S) (12) • Ca enhances the detection of potassium (K) and simultaneously K can absorb characteristic photons produced by Ca, inhibiting its detection (12) • Ca will enhance the detection of Mercury (Hg) "even in normal concentrations" (12) • Ca will excite lead (Pb) making Pb concentration seem higher (12)
Iron (Fe)	• High Fe concentration can enhance the detection Ca (12,39) • Zn can enhance the detection of Fe (5) • Nickel (Ni) can enhance the detection of Fe (44) (as Ni is present in pXRF instruments, this may have a constant effect) • Fe can enhance the detection of chromium (Cr)
Zinc (Zn)	• Fe can absorb characteristic photons from Zn, inhibiting its detection (5)
Copper (Cu)	• Cu absorbs characteristic photons created by Pb (51)
Rhodium (Rh)	• Rh may excite tungsten (W), mercury (Hg), thallium (Tl), Pb, bismuth (Bi) and S making them appear more abundant (12). As Rh may be used as the X-ray source in many pXRF instruments, this may have a constant effect
Lead (Pb)	• Pb does not allow photons to escape the matrix so its presence in the sample can inhibit the detection of all elements (12) • Pb fluorescence is boosted by rhodium (Rh) tube devices and may overwhelm the emission peaks from other elements (12)

2.3.7 Common spurious peaks and XRF for museum zoological specimens

The analysis of elemental data from an XRF spectrum begins with the identification of each peak and can be complicated by the presence of spurious peaks including escape and sum

peaks. These may be falsely identified as characteristic peaks of elemental components in the sample or may overlap elemental emission lines increasing their intensity (11).

Sum peaks spontaneously occur when two fluorescence photons hit the surface of the detector at the same moment, both photons are counted as one and their energies co-added, resulting in a high energy peak in the spectrum (39). This can occur with any two characteristic photons but is more common with those emitted from the inner shell of an atom (K-shell emissions) as they are more abundant in the overall fluorescence from the sample (52).

Escape peaks arise when the detector itself is excited by the incoming photons from the sample causing fluorescence from the elements within the detector and reducing the energy of that incoming photon (37). For instruments equipped with silicon (Si) drift detectors, the characteristic photons are reduced by the energy of Si (1.74 keV).

Both sum and escape peaks are more common when elemental concentrations are high (53), and the literature suggests that high elemental concentrations may be encountered in some zoological museum specimens (54,55). The intensity of sum peaks may be reduced by lowering the power to the X-ray tube, thereby reducing the intensity of the X-ray incident beam. However, the use of a filter is reported to reduce the incidence of numerous spurious peaks in museum specimens, including sum peaks, escape peaks and Bragg scatter, by selectively filtering the incidence X-ray beam (11,56). While Bezur recommended the use of a three-part filter (25 μm Ti/300 μm Al/75 μm Cu) to improve resolution of heavy metals in historic zoological specimens (44), this study aimed to observe a broader number of elements and the inclusion of the thicker Cu layer would reduce the detection of lower atomic weight elements (11). Therefore, a 25 μm Ti/300 μm Al filter was selected.

Less predictable, but equally misleading, are peaks that appear in response to X-ray interactions with atoms. Rayleigh scatter occurs in most sample types, when the incident radiation beam is reflected by compositional atoms without loss of energy, producing peaks characteristic of the instrument's radiation tube. Some elements will produce higher Rayleigh scatter than others. Satellite lines, which occur when a second electron is ejected, can appear spontaneously in a spectrum. Finally, the incidence of the Auger effect, and consequent peaks, is high in samples with an abundance of low Z weight elements like skin (4).

2.4 XRF peak attributions for preserved skin

The capacity to correctly identify each peak within the spectrum is influenced by operator experience (53). Spectral interpretation is typically based upon tables of X-ray fluorescent lines for each element (56) and these may be built into instrument software. Few publications have included XRF spectra produced by natural history specimens, and the interpretation of overlapping emission lines and spurious peaks are rarely discussed in case studies. Overlapping emission lines have the potential to complicate spectral interpretation (12,56). While these may be partially overcome by peak evaluation algorithms in the instrument software, where concentrations are high or analytes include heavy metals, it is recommended to rely solely on instrument software (5,11). For zoological (and ethnographic) museum collections, preservatives and pesticides that include Pb, As, Hg, and Br may be found alone or in combination, creating considerable difficulty in interpretation (39).

This section documents the interpretation of pXRF spectra produced by skin standards treated with a selection of preservatives recorded in 18th and 19th century manuals on natural history specimen preservation. A summary of the mitigation strategies applied in the protocol are outlined in Table 2.2. Skin standards were created from the belly region of the one animal, a pig. Blanks and untreated skins samples were interrogated to identify peaks that arose from the

instrument and from skin. Each spectrum captured the elemental composition of both skin and preservative chemicals, and the spectral features arising from mixtures of preservatives in a skin matrix. Samples treated with preservatives were compared to untreated skin to identify characteristic peaks.

Table 2.2: Summary of mitigations applied

Matrix factors affecting pXRF spectroscopy of historic museum specimens	**Experimental procedures to mitigate matrix factors**
Irregular sample geometry including convex and concave surfaces	• Study skins which typically have some relatively flat surfaces, were selected for analysis where possible • Measurements collected from numerous sites on each specimen that do not overlap allowing for rejection of measurements with very low counts • Sites selected for data collection are visibly as flat as possible
Presence of fur layer and variation in fur density across single animals and between animals	• Measurements collected from numerous sites on each specimen that do not overlap • Normalisation of spectra to Compton peak area • Custom mount - Specimen rests above the nose of instrument effectively compressing fur layer under the weight of the specimen itself • Limited variation in species selected and using mammals not birds to minimise impact of air gaps • Cautious consideration of count numbers for low Z weight elements • In calculating median values, a similar number of data measurement sites were used for each specimen to account for intra specimen variability
Variation in skin thickness and density across single	• Numerous measurements • Normalisation of spectra to Compton peak area • Limited variation in species selected

animals and between animals	• Cautions interpretation of results for low Z weight elements
High Bremsstrahlung scatter produced by biological samples	• 25 µm Ti - 300µm Al filter used in data collection to reduce continuum scatter and improve interpretation of the spectra
High elemental concentration producing spurious peaks	• 25 µm Ti - 300 µm Al filter used in data collection filter incident X-ray beam
Influence of internal structures on XRF data	• Careful selection of measurement sites to reduce influence of internal structures • Removal of outlier measurements • Numerous measurements
High contribution of surface layer treatments to overall fluorescence measurement	• Measurements collected from numerous sites on each specimen that do not overlap to identify both highly variable elements, and those that are consistently present • Interpreting the standard deviations of the elemental data as indicative of preservative/pesticide application method • Significant outliers within the data were considered a result of surface contamination of the specimen, and removed
Moisture effects	• Cautious comparison of specimens housed in significantly different environmental conditions, museums or storage areas
Variation in crystal sizes of preservatives	• 8 mm collimator fitted to the instrument to enable largest spot size for data collection • Numerous measurements • Removal of outlier measurements
Chemical matrix effects	• Awareness of chemical effects on interpreting the XRF data • Observation of XRF interactions between elements in the coming section
Interpretation of XRF data	• Semi-quantitative net peak area has been used to derive relative quantities and ratios • Peak allocations for standards to identify likely spurious peaks (see Section 2.4)

2.4.1 Experimental

2.4.1.1 Materials – reference standards

Reference standards were made from a single piece of food grade *Artiodactyla Sus domesticus* (pig) skin. Pig skin was selected because it was readily obtainable, and it retained physical integrity during preliminary evaluation of tolerance to handling and physical impacts of repeated analytical tests compared to poultry skin. It had clearly identifiable dermal and epidermal layers, allowing for straightforward identification of the inner flesh side and outer hair side.

The pig skin, taken from the belly section, was obtained from a fresh meat supplier, Ben's All Meats in Belconnen, ACT. The animal was grown in Australia and processed at an Australian abattoir according to national food standards (57). The skin section was prepared by manually scraping the flesh side with a stainless-steel knife to remove as much fat as possible. Standards were cut to approximately 10 mm × 10 mm with a thickness of 0.8-1.2 mm. These samples were soaked in 100% ethanol (undenatured) for 12 hours to represent wet preservation in 'spirits of wine'. The ethanol, having become cloudy was replaced after 12 hrs. Standards, selected at random, were air dried before the application of preservative chemicals. Three replicates were prepared for each preservative.

Dry preservatives were first ground into a fine powder using a mortar and pestle. This was then rubbed into the flesh side of the skin with gloved hands. To represent the distribution of wet preservatives via brush, liquid preservatives were applied via glass pipette and distributed over the flesh side surface using a glass rod (Fig. 2.9). A table of preservative solutions and application methods is provided below (Table 2.3). After application, each standard was placed in a labelled, glass vial. Standards were heated at 50 °C for 7 hrs within a fume hood, using a

heating plate. After cooling, standards were housed, with their replicates, in glass vials with airtight polyester lids and stored in a dark room temperature controlled to 22 ± 3 °C.

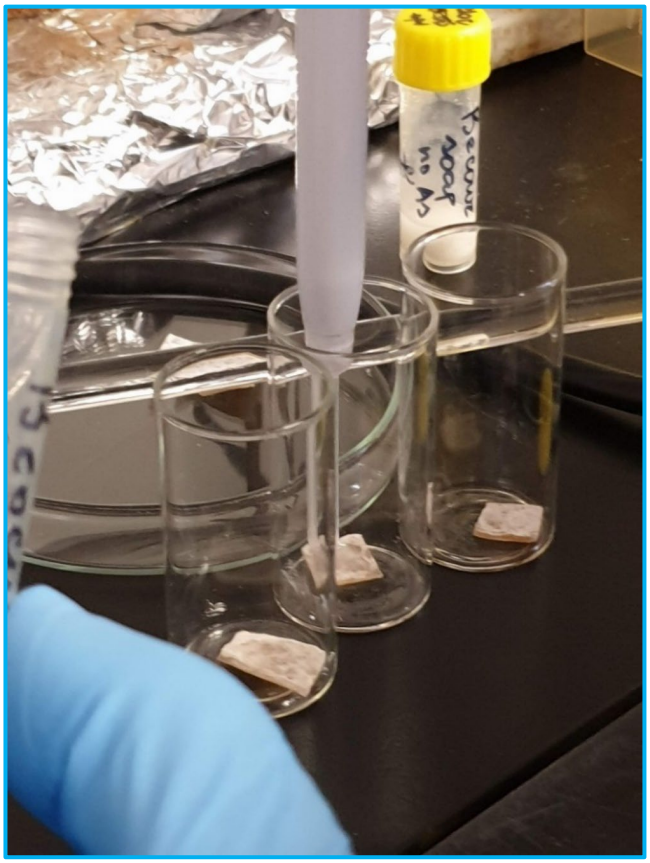

Fig. 2.9: Photograph shows the application of arsenical soap to pig skin reference samples.

2.4.1.2 Materials - historic specimens

With the approval of the Museum, physical samples of approximately 10 × 10 mm, were removed from two mounted specimens from the Macleay Collection, The University of Sydney (MAMU) via scalpel. Both samples were 0.5-0.8 mm thick, which was thinner than the reference standards. These were housed in individual zip-lock bags and stored in a dark room temperature controlled to 22 ± 3 °C with the reference standards.

Table 2.3: Reference standards of pig skin treated with common 18th and 19th century preservatives

Preservative name	CAS	Historic names	Application method	ICP-MS digestion
Untreated (Control)				HNO_3 + plastic vial HCl + glass vial
Ammonium Chloride - NH_4Cl	12125-02-9	Sal ammoniac*, muriate of ammonia*, sal aremonicum*	Applied as powder rubbed into skin	HNO_3 + plastic vial
Zinc chloride - $ZnCl_2$	7646-85-7	Chlorinate of zinc*, Burnett's disinfecting solution,	Applied in solution with H_2O via pipette	HNO_3 + plastic vial
Mercury(II) chloride - $HgCl_2$	7487-94-7	Mercury sublimate, mercurous chloride, calomel**, corrosive sublimate**	Applied as a saturated solution in H_2O via pipette + distributed using glass rod	HCl + glass vial
Potassium aluminium sulfate - $KAl(SO_4)_2$	7784- 24-9	Alum, potash alum, native alum*, dry alum ***	Applied as powder rubbed into skin	HNO_3 + plastic vial
Ammonium aluminium sulfate - $(NH_4)Al(SO_4)_2$	7784-26-1	Ammonium alum, just alum	Applied as powder rubbed into skin	HNO_3 + plastic vial
Potassium sulfate - K_2SO_4	7778-80-5	Potash, sulphate of potash*, Sthal's sulphureous*	Applied as powder rubbed into skin	HNO_3 + plastic vial
Iron(II) sulfate - $FeSO_4$	13463-43-9	Green vitriol*, ferrous sulphate, English vitriol*, green copperas*	Applied via solution in H_2O via pipette	HNO_3 + plastic vial
Potassium nitrate - KNO_3	7757-79-1	Saltpetre, halinitrum*, niter, sal prunella*	Applied as powder rubbed into skin	HNO_3 + plastic vial
Lead(II) nitrate - Pb(NO3)2	10099-74-8	Analogue for lead acetate Plumbum dulce*, sweet lead*, Goulard water**, sugar of lead**	Applied as a slurry in H_2O via pipette + distributed using glass rod	HNO_3 + plastic vial
Arsenic trioxide - As_2O_3	1327-53-3	White arsenic, arsenous acid*, white alum*,	Applied as a slurry in H_2O via pipette + distributed using glass rod	HNO_3 + plastic vial
Arsenical soap made up of; • Sodium hydroxide NaOH • Sodium sulfide nonahydrate $Na_2S{\cdot}9H_2O$ • Arsenic trioxide As_2O_3 • Potassium stearate $C_{18}H_{35}KO_2$ • Camphor $C_{10}H_{16}O$	1310-73-2 1313-84-4 1327-53-3 593-29-3 76-22-2	Similar to Dufresne's recipe for arsenical soap (58,59).	Spontaneously formed a thick paste, applied via pipette + distributed using glass rod	HNO_3 + plastic vial
Mixture of: • Lead(II) nitrate $Pb(NO_3)_2$ • Arsenic trioxide As_2O_3	10099-74-8 1327-53-3	Elemental analogue of lead arsenate	Applied (in layers) as a slurry in H_2O via pipette. Allowed to dry between layers	HNO_3 + plastic vial HCl + glass vial
Mixture of; • Mercury(II) chloride $HgCl_2$ • Lead(II) nitrate $Pb(NO_3)_2$ • Arsenic trioxide As_2O_3 • Aluminium sulfate $Al_2(SO_4)_3$ • Iron(II) sulfate $FeSO_4$	487-94-7 10099-74-8 1327-53-3 10043-01-3 13463-43-9		Applied (in layers) as a slurry in H_2O via pipette + distributed using glass rod. Layers allowed to dry between applications	HNO_3 + plastic vial HCl + glass vial

*(60-62), **(63)

Table 2.4: Specimens from the MAMU collection

Accession number	Species and remnant specimen data	Description	ICP-MS digestion
NHM457	Rufous Bettong - *Aepyprymnus rufescens* New South Wales, Australia Collected c. 1870. Associated with Sir William John Macleay of Elizabeth Bay, Sydney	Mounted mammal specimen. Internal support of wire wrapped in two fibres. Losses to ears and displaced front limb. Repairs made to right limb using iron wire and cotton gauze. Sample is dark tan in colour. Flesh side has raised white patches randomly distributed. Black and bright orange particulates embedded across flesh side surface.	HNO_3 + plastic vial HCl + glass vial
NHB1354	Red winged parrot - *Aprosmictus erythropterus* Moreton Bay, Queensland, Australia Collected c. 1870s. Associated with Sir William John Macleay of Elizabeth Bay, Sydney	Mounted bird specimen in poor condition with large section of breast lost and cotton stuffing visible. Skin sample is light in colour with fine black spots and planar delamination on flesh side.	HNO_3 + plastic vial HCl + glass vial

2.4.1.3 XRF spectroscopy

Analysis was undertaken using a Bruker Tracer 5i portable XRF spectrometer (heretofore referred to as Tracer), with a Rh thin-window X-ray tube; X-ray generator 6-50 kV (4.5-195 μA, maximum 4 W output), and a 20 mm^2 silicon drift detector with 140 eV @ 250,000 cps Mn K_α; resolution. The instrument was fitted with an 8 mm collimator and controlled via a laptop using Bruker ARTAX software (v8.0.0.476). As per requirements under NSW (Australia) state regulations, the operator was certified to use portable radiation emitting instruments.

A 25 µm Ti - 300 µm Al filter was selected to minimise Bragg scattering and escape peaks and samples were analysed in air at 40 kV as suggested by Shugar and Sirios (11). Power was set to 30 µA. A 40 s measurement time was selected after time trials were undertaken to determine the optimal measurement acquisition time (see Appendix 1). At least three spectra were collected from each standard and these were co-added using the accumulate function in the Bruker ARTAX software (v8.0.0.476). Spectra were observed and deconvoluted using the same software.

2.4.1.4 Inductively coupled plasma - mass spectrometry (ICP-MS)

Two digestions were undertaken followed by analysis using a PerkinElmer NexION 300X ICP Mass Spectrometer. The NexION results were calculated using Syngistix for ICP-MS version 1.1 (PerkinElmer Inc., Shelton, CT). These results were then exported to Excel and factored concentrations were derived based on the dilution factor and the initial weight of each sample.

The first digestion included all reference standards and museum specimens. Small samples were cut from each using a stainless-steel blade. These were placed in plastic vials with 1 mL of 70% concentrated nitric acid (HNO_3) and were allowed to digest in a fume hood for 16 hrs at approximately 25 °C. Each vial was then mixed in a vortex mixer for 8 s and spun in a benchtop centrifuge at 1600 rpm for 10 s. Digestion fluid (100 µL) was diluted to 10 mL with millipore water. Three replicates of each digestion were analysed using a 54 Multi element mix (certificate provided in Appendix 2) plus mercury. This experiment was performed in a fume hood. All participants used protective clothing including latex gloves, eye protection and washable lab coats.

Strekopytov *et al*., noted that Hg can volatise when digested in HNO_3 (55), thus a second digestion was undertaken focusing on obtaining comparative results for Hg using hydrochloric acid (HCl). As before, a small sample was cut from each reference standard that included Hg

and from both museum specimens. These were placed in glass vials with 1 mL of 70% concentration HCl and thereafter treated in the same manner as the HNO_3 digestion. An average result for each standard was calculated and these are documented in Appendix 3.

2.4.2 Results – reference standards

Spectra were produced and peaks attributed for each reference sample and for the two historic museum specimens. The following pages document the results and discussion of XRF spectra produced by;

- Blank
- Untreated skin reference (Control)
- Potassium sulfate treated skin reference - K_2SO_4
- Ammonium aluminium sulfate treated skin reference - $(NH_4)Al(SO_4)_2$
- Potassium aluminium sulfate treated skin reference - $KAl(SO_4)_2$
- Lead(II) nitrate and arsenic trioxide treated skin reference - $Pb(NO_3)_2 + As_2O_3$
- Lead(II) nitrate, arsenic trioxide, mercury chloride, aluminium sulfate and iron(II) sulfate treated skin reference - $Pb(NO_3)_2$, As_2O_3, $HgCl_2$, $Al_2(SO_4)_3$, $FeSO_4$
- NHM457, rufous bettong (*Aepyprymnus rufescens)*
- NHB1354, red winged parrot (*Aprosmictus erythropterus)*

Discussion for the following spectra can be found in Appendix 4.

- Ammonium chloride treated skin reference - NH_4Cl
- Zinc chloride treated skin reference - $ZnCl_2$
- Mercury chloride treated skin reference - $HgCl_2$
- Iron(II) sulfate treated skin reference - $FeSO_4$
- Potassium nitrate treated skin reference – KNO_3
- Arsenic trioxide treated skin reference - As_2O_3

- Lead(II) nitrate treated skin reference - $Pb(NO3)_2$

2.4.2.1 Blank

To assess background peaks produced by the instrument itself, the pXRF was run three times without a sample above the window, and with the proximity sensor safety feature disabled, see spectrum in Fig. 2.10.

An argon (Ar) peak at 2.95 keV was attributed to air. Tin (Sn) peaks at 3.44, 3.66, and 25.30 keV were attributed to the radiation shield at the nose of the instrument (12). The hafnium (Hf) peak at 7.89 keV and tungsten (W) peak at 8.40 keV were attributed to the radiation shielding alloys (64). Peaks for aluminium (Al) at 1.49 keV, Fe at 6.39 keV, Ni at 7.47keV, Cu at 8.04 keV, and Zn at 8.62 keV were attributed to the detector case, collimator, and the tube lens mount (12,52). Cr peaks at 5.42 and 5.95 keV and Ti peaks at 4.50 and 4.93 keV were also identified. Both Compton (incoherent) and Rayleigh (coherent) scatter peaks are observed between 18.5 and 23.0 keV respectively.

2.4.2.2 Untreated skin reference (control)

The spectrum of the control, pig skin prepared with ethanol but not treated with any further preservatives, is shown in Fig. 2.11. Characteristic peaks were identified for S (2.30 keV), Cl (2.62 keV), K (3.32keV), Ca (3,69 and 4,01 keV), Fe (6.40 and 7.04 keV), Cu (at 8.04 and 8.91 keV), and Zn (8.63 and 9.57 keV). These results are in agreement with the literature (65). A small peak for rubidium (Rb) was identified at 13.39 keV, which may be expected as a trace element in skin (66).

Characteristic peaks were identified for Bromine (Br) at 11.92 and 13.32 keV. While Br is not often mentioned as a component of skin, McCall *et al.,* stated that Br is essential for collagen IV scaffolds (67). While skin is predominantly collagen I and III, collagen IV is also present

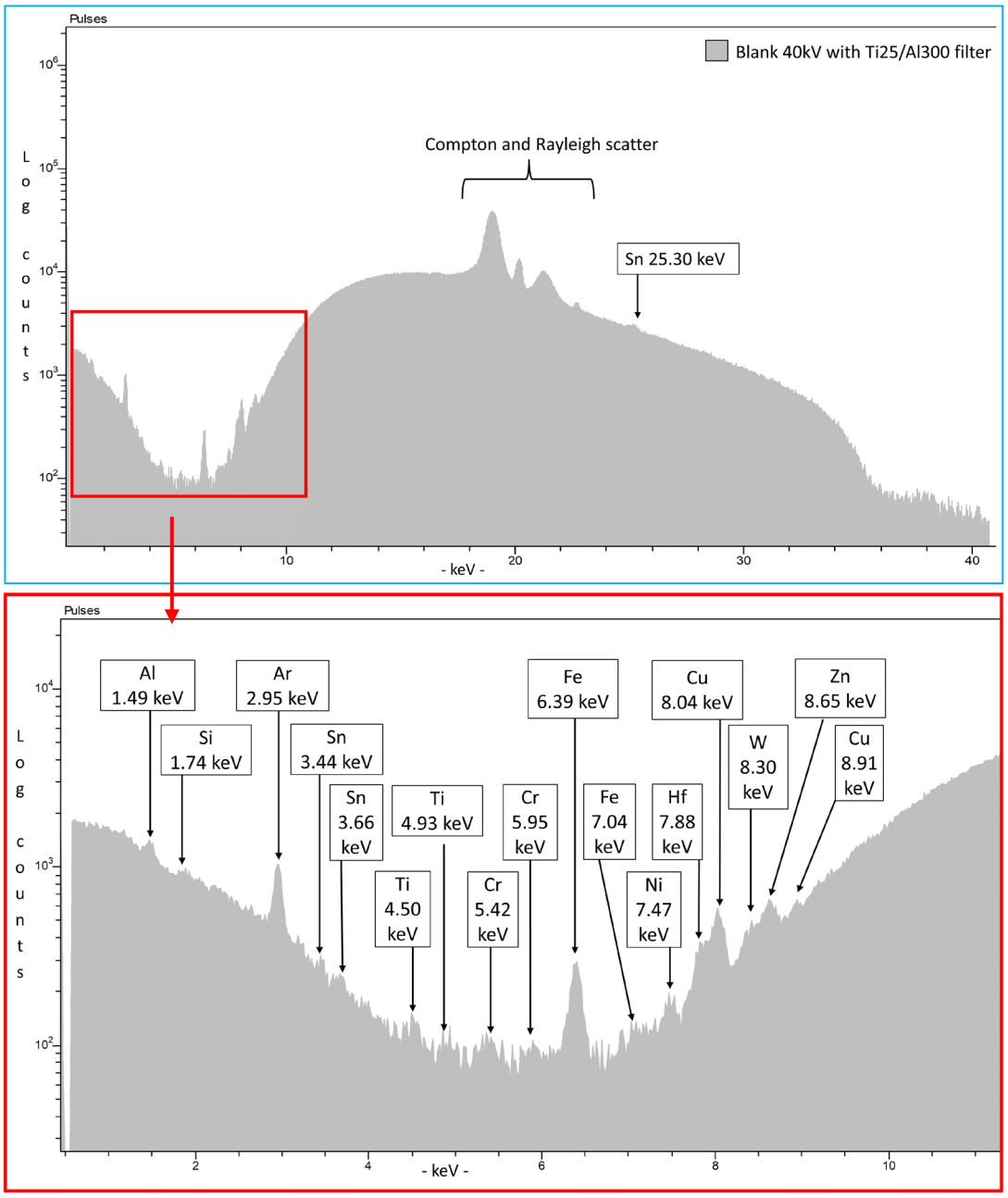

Fig. 2.10: "Blank" XRF spectrum collected without a sample to identify instrument related peaks. Configuration settings 40 kV, 30 µA, and 30 s using a 25 µm Ti - 300 µm Al filter.

(68). Ceko et al., found high levels of Br in cattle tissues using XRF and attributed its presence to exposure to agricultural pesticides and herbicides, such as potassium bromide and methyl bromide, or to processing disinfectants containing dibromoacetic acid (69). Australian food standards show that the disinfectants bromo-chloro-dimethylhydantoin and dibromo-

dimethylhydantoin may be used as a washing agent, and this may be a source of Br beyond that naturally occurring in skin (57). Both Compton (incoherent) and Rayleigh (coherent) scatter peaks are observed between 18.5 and 23.0 keV.

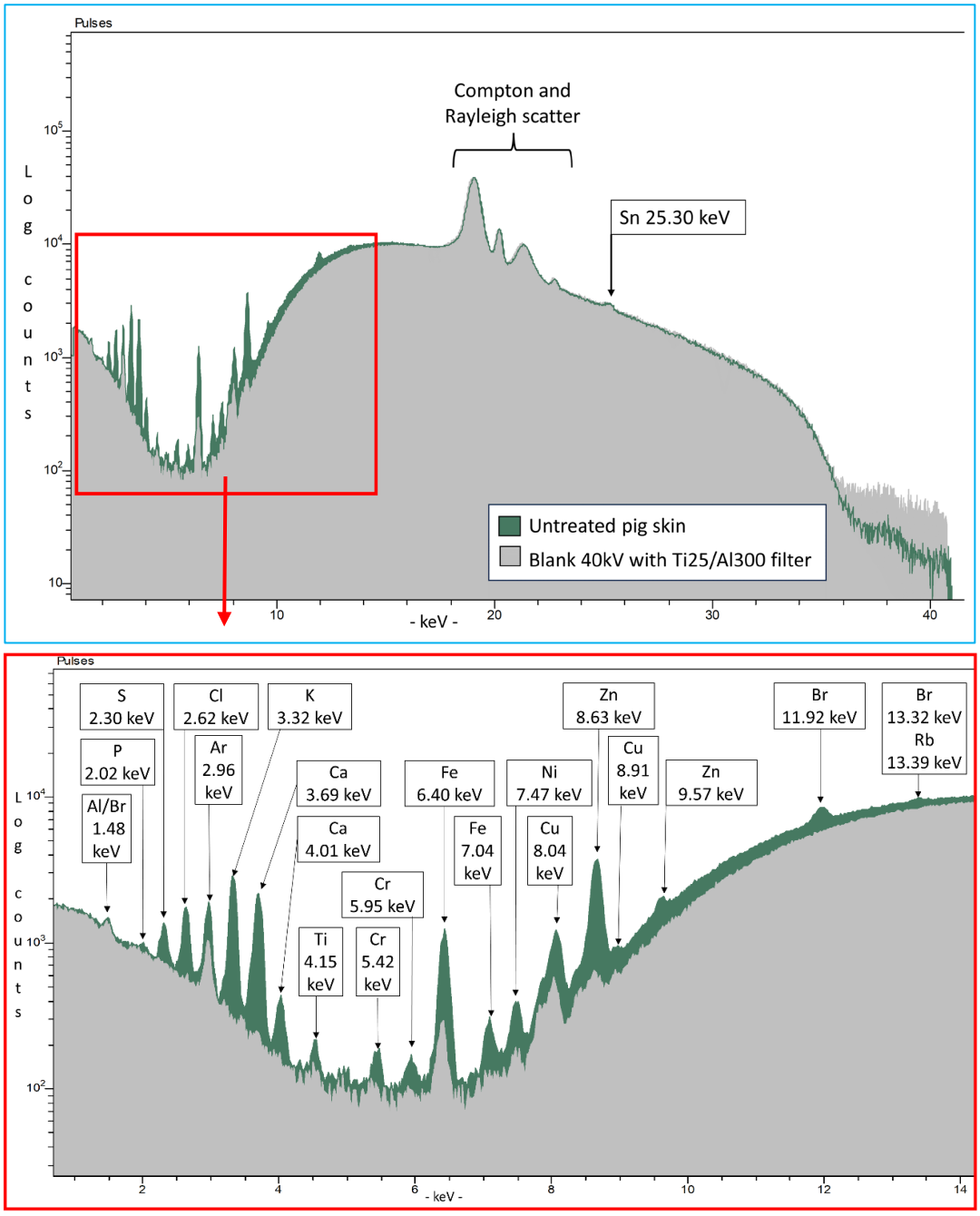

Fig. 2.11: XRF spectrum collected from three untreated pig skin standards and co-added using software accumulate function (green), overlayed with blank spectrum (grey). Spectra have been normalised to the Compton peak for comparison.

2.4.2.3 Potassium sulfate treated skin reference - K_2SO_4

In Fig. 2.12, characteristic peaks for K were identified at 3.31 (K K_α) and 3.58 keV (K K_β), and peaks attributed to S were identified at 2.30 (S K_α) and 2.46 keV (S K_β). Escape peaks related to K K_α and K_β fluorescence were identified at 1.56 and 1.84 keV, respectively. The K K_α escape peak at 1.56 keV partially overlapped the characteristic peak for Al K_α (1.48 keV), resulting in artificially high counts for Al when peak area evaluation was run via instrument software. A sum peak from K K_α (3.31 + 3.31 keV) was observed at 6.60 keV. A weak feature of elevation around 5.76 keV was attributed to sum peaks arising from S K_β and K K_α (2.46 + 3.31 keV).

2.4.2.4 Ammonium aluminium sulfate treated skin reference - $(NH_4)Al(SO_4)_2$

Fig 2.13 shows the spectrum from $(NH_4)Al(SO_4)_2$ treated skin. Characteristic peaks for S were identified at 2.30 and 2.46 keV (S K_α and S K_β, respectively). It was noted that no significant change in the intensity of this Al/Br peak at 1.48 keV was observed in this spectrum.

2.4.2.5 Potassium aluminium sulfate treated skin reference - $KAl(SO_4)_2$

Fig 2.14 shows the spectrum collected from skin treated with $KAl(SO_4)_2$ in light blue. Characteristic peaks for K K_α and K K_β were identified at 3.31 and 3.58 keV respectively, and peaks attributed to sulfur were identified at 2.30 and 2.46 keV (S K_α and S K_β respectively).

Like the spectrum collected from skin treated with $(NH_4)Al(SO_4)_2$ in Fig. 2.13, treatment with $KAl(SO_4)$ did not produce increased intensity of the Al K_α peak at 1.48 keV. The ICP-MS results determined that the concentration of Al in the $KAl(SO_4)$ sample was 83.68 ppm and the $(NH_4)Al(SO_4)_2$ treated sample had an Al concentration of 103 ppm. Both significantly higher than the untreated sample at 1.31 ppm and the sample treated with K_2SO_4, which had an Al concentration of 0.13 ppm (Fig. 2.15). In all cases, the intensity of the Al K_α peak did not reflect the variation in Al concentration in these samples which demonstrated that the detection of

fluorescence produced by Al was significantly inhibited by air attenuation and matrix effects associated with these skin samples.

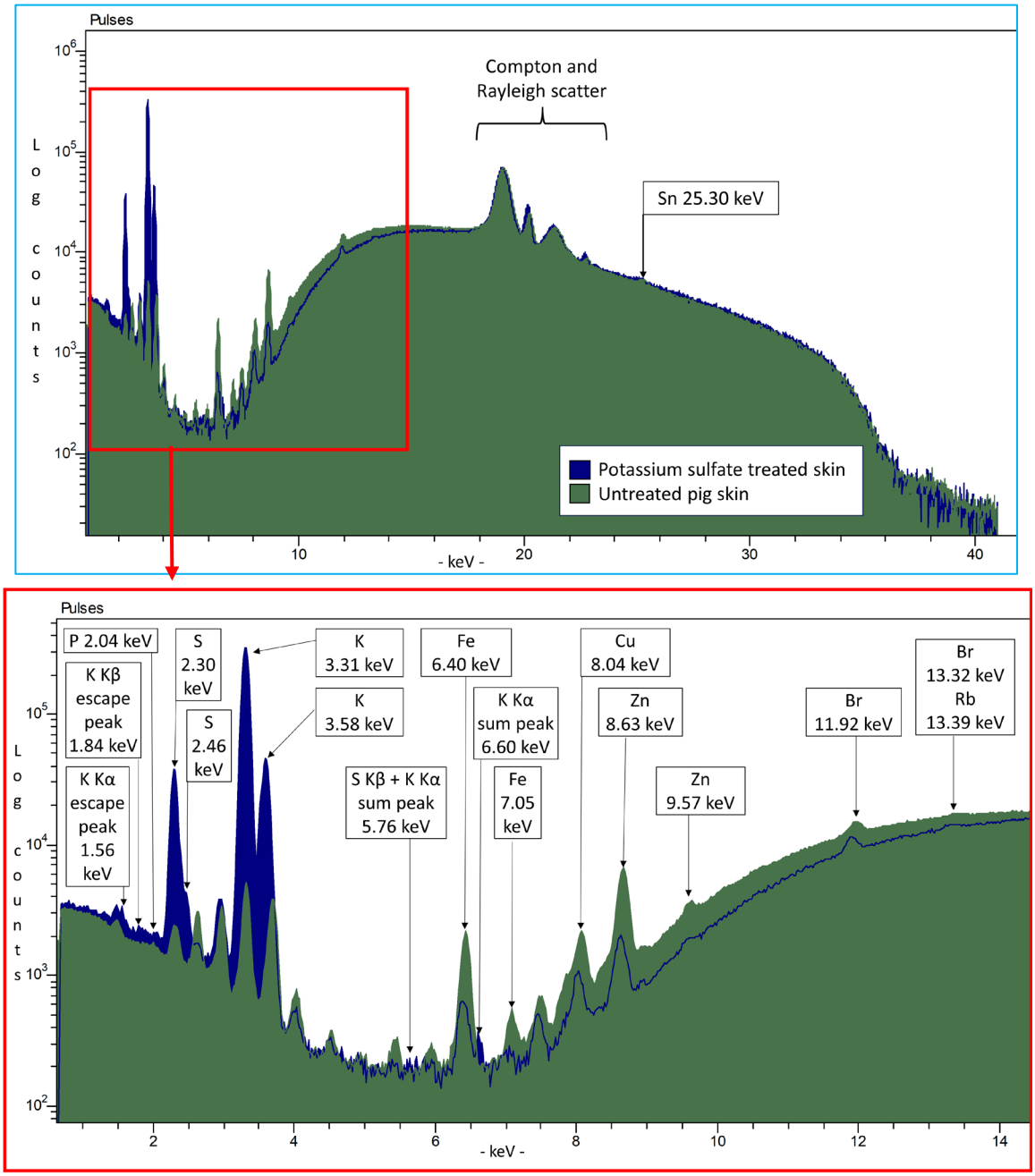

Fig. 2.12: XRF spectrum collected from three reference standards treated with potassium sulfate and co-added using software accumulate function (navy blue), overlaid with a spectrum collected from three untreated skin standards, also co-added. Spectra have been normalised to the Compton peak for comparison.

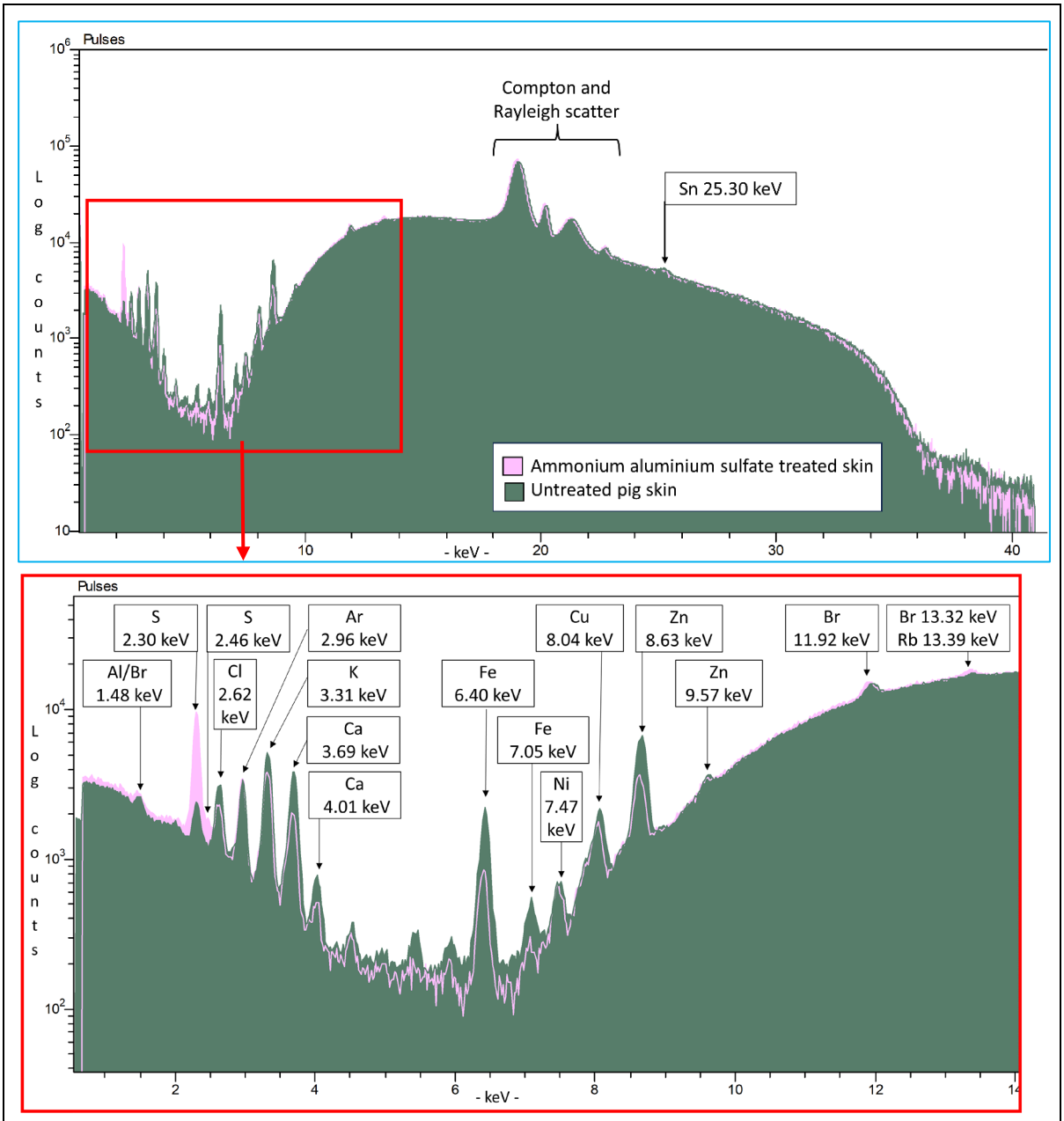

Fig. 2.13: XRF spectrum collected from three reference standards treated with ammonium aluminium sulfate and co-added using software accumulate function (pale pink), overlaid with a spectrum collected from three untreated skin standards, also co-added. Spectra have been normalised to the Compton peak for comparison.

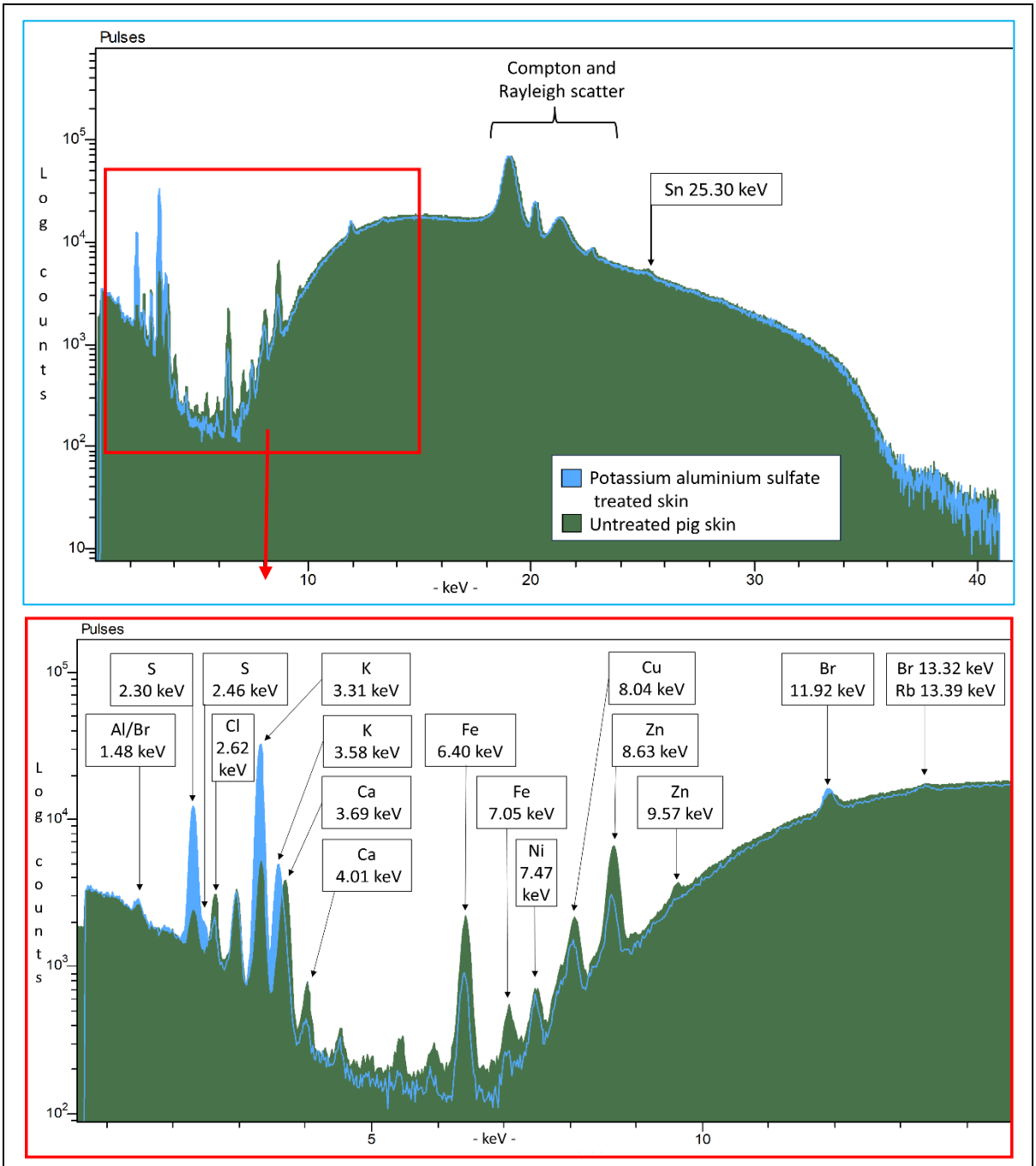

Fig. 2.14: XRF spectrum collected from three reference standards treated with potassium aluminium sulfate and co-added using software accumulate function (light blue), overlaid with a spectrum collected from three untreated skin standards, also co-added. Spectra have been normalised to the Compton peak for comparison.

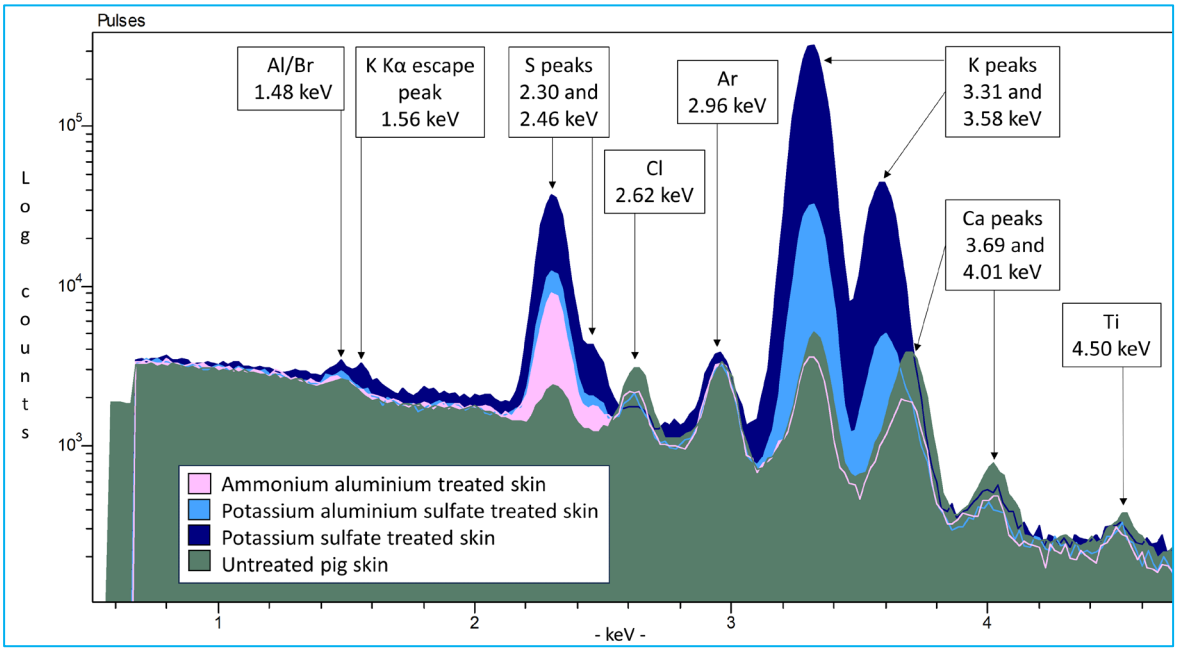

Fig. 2.15: The 0.50 – 4.50 keV region showed little variation in the intensity of the Al K_α peak at 1.48 keV regardless of varying amounts of Al present in the samples. Spectra were collected from samples treated with potassium sulfate (navy blue), ammonium aluminium sulfate (pale pink), potassium aluminium sulfate (light blue), and untreated samples (dusty green). All spectra have been normalised to the Compton peak for comparison.

2.4.2.6 Lead(II) nitrate and arsenic trioxide treated skin reference - $Pb(NO_3)_2 + As_2O_3$

The spectrum produced by skin treated with both $Pb(NO_3)_2 + As_2O_3$ is presented in Fig. 2.16. Characteristic As peaks were identified at 10.53 keV (As K_α) and 11.73 keV (As K_β). With this combination of preservatives, As K_α is completely overlapped by Pb L_α (1 and 2) emission lines. Only the As K_β peak can be used to confirm the presence of this element in the sample. Characteristic peaks for Pb were identified at;

- 1.84 keV = Pb M_ζ
- 2.39 keV = Pb M_5 transition to O_3 transition plus M_α and M_β emissions. This overlapped S K_α and K_β at 2.30 and 2.46 keV
- 2.63 keV = Pb M_γ which overlaps Cl K_α at 2.62 keV

- 3.11 keV = Pb M2 transition to N4
- 9.17 keV = Pb L_{ι}
- 11.35 keV = Pb L_{η}
- 12.30 keV = Pb $L_{\beta 4}$
- 12.62 keV = Pb L_{β} (1 and 2)
- 13.00 keV – Pb $L_{\beta 5}$
- 14.30 keV = Pb L_{γ}
- 14.76 keV = Pb $L_{\gamma 1}$
- 15.16 keV = Pb $L_{\gamma 6}$, $L_{\gamma 2}$, $L_{\gamma 3}$ this overlaps Rb K_{β} at 15.19 keV
- 15.75 keV = Pb L_{γ}

An escape peak at 1.31 keV was attributed to the Pb M_3-$O_{4,5}$ transition (3.05 -1.74 keV), and an escape peak arising from As K_{α} was observed at 8.79 keV (10.53 – 1.74 keV). Further spurious peaks were identified at high energies. Enhancement of Rh peaks at 20.17 and 22.70 keV are from enhanced Rayleigh scatter. A sum peak at 21.10 keV was attributed to the sum of Pb L_{α} (10.55+10.55 keV) and may also be influence by a sum peak from As K_{α} sum peak (10.53 + 10.53 keV). At 22.26 keV a sum peak for As K_{α} + As K_{β} was observed (10.53 + 11.73 keV). A sum peak for Pb L_{η} at 22.68 keV (11.34 +11.34 keV) overlapped the Rayleigh peak for Rh at 22.72 keV modifying the shape of the peak. At 23.16 keV, a sum peak for Pb L_{α} + Pb L_{β} (10.55+12.62 keV) was identified, and a sum peak arising from the sum of Pb L_{β} and As K_{β} emissions was found at 24.33 keV. An additional sum peak arising from Pb $L_{\beta 1}$ was identified at 25.24 keV (12.62+12.62 keV), which overlapped Sn K_{α} at 25.30 keV, and another peak at 27.37 keV was attributed to the sum of Pb L_{γ} and Pb L_{β} emissions (14.75+12.62 keV).

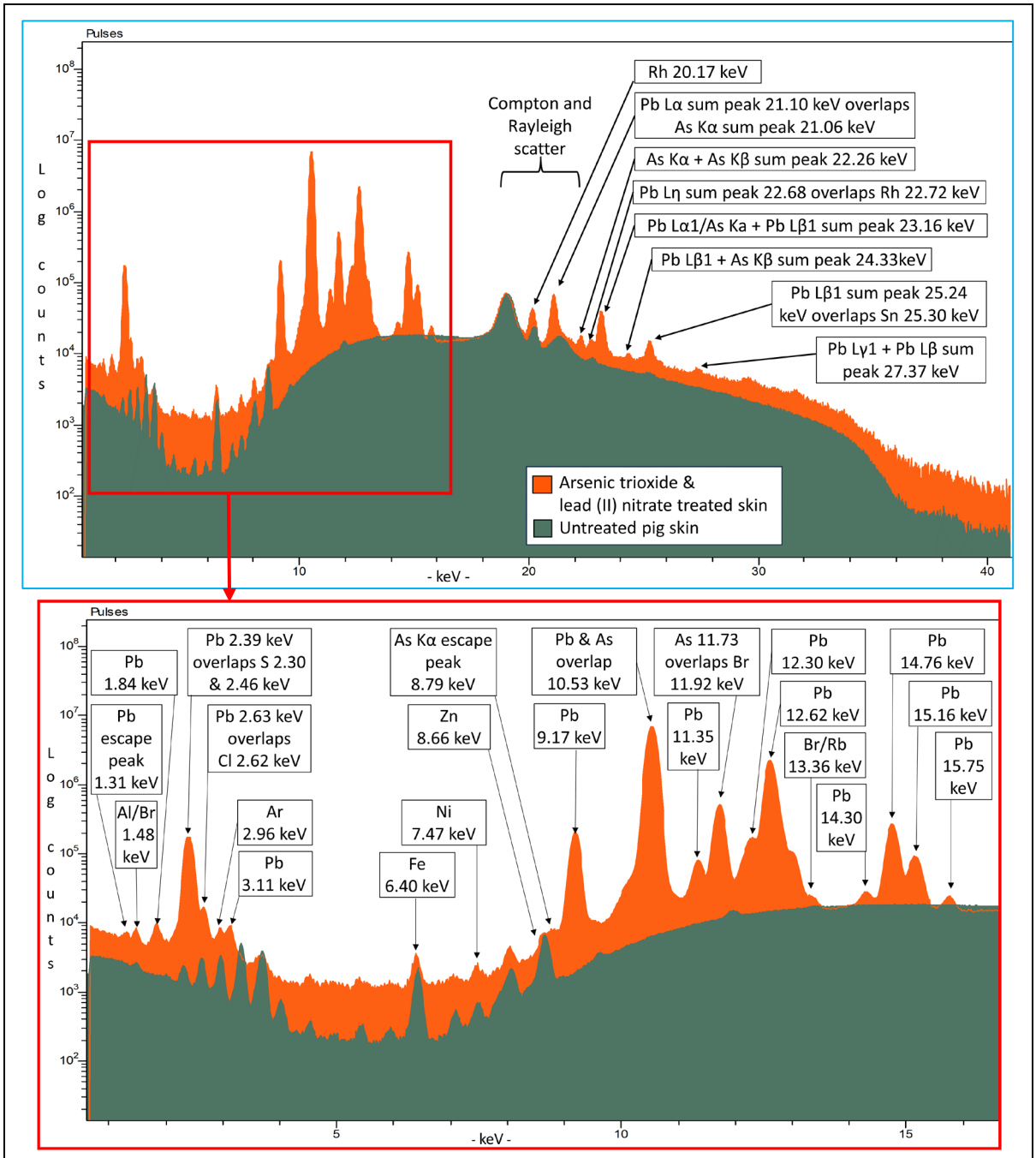

Fig. 2.16: XRF spectrum collected from three reference standards treated with arsenic trioxide and lead(II) nitrate, co-added using software accumulate function (orange), and overlaid with a spectrum collected from three untreated skin standards. Spectra have been normalised to the Compton peak for comparison.

2.4.2.7 Lead(II) nitrate, arsenic trioxide, mercury chloride, aluminium sulfate, and iron(II) sulfate treated skin reference - $Pb(NO_3)_2$, As_2O_3, $HgCl_2$, $Al_2(SO_4)_3$, $FeSO_4$

This reference standard was prepared to explore the deconvolution of the XRF spectrum produced by mixtures of preservatives, see Fig. 2.17. The combination of Pb, As, and Hg is commonly found in museum zoological specimens. Al and Fe were included to represent the alum and green vitriol respectively.

It was shown in Section 2.4.2.5, that Al is difficult to detected in the pig skin standards using this protocol. Consequently, the enhancement of the peak at 1.48 keV was attributed to As L_γ at 1.52 keV. The broad peak at 2.30 keV was attributed to the combination of S K_α at 2.30 keV, Pb M_α at 2.34 keV, S K_α at 2.46 keV, and Hg M_β at 2.28 keV. The less intense peak at 2.63 keV was attributed to Pb M_γ which overlaps Cl K_α at 2.62 keV. The secondary Cl K_β at 2.82 keV is no longer identifiable. The intensity of the peaks at 3.31 keV (attributed to K K_α) and 3.69 keV (attributed to Ca K_α) were both reduced. This was similar to data from the standard treated only with $Pb(NO_3)_2$ (Appendix 4).

The broad, low intensity peak at 4.66 keV was an escape peak from Fe K_α (6.40-1.74 keV). The nearby 5.88 keV peak was identified as Mn K_α originating from the contaminated $FeSO_4$ (identified in Section 2.4.2.9), and overlapped with the instrument Cr K_β (identified in Section 2.4.2.1). Fe K_α peak at 6.40 keV and Fe K_β at 7.05 keV were identified. The Zn K_α peak at 8.63 keV was overlapped and intensified by Hg L_ι at 8.72 keV. The Zn K_β peak at 9.57 keV is masked by peaks on either side from Pb L_ι at 9.17 keV and Hg L_α at 9.98 keV. Interestingly, the Hg L_α peak (at 9.98 keV) and the Pb L_ι peak (at 9.17 keV) were not overlapped by any other features, making these peaks suitable for qualitative and semi-quantitative assessments in museum specimens.

The peak at 10.53 keV was attributed to As K_α but is almost entirely overlapped by Pb L_α at 10.54 keV and Hg L_η at 10.64 keV. The As K_β peak at 11.73 keV is also overlapped, this time by Hg L_β at 11.82 keV. This made As identification difficult when both Hg and Pb are present in the sample. The As L_γ at 1.52 keV was helpful for qualitative purposes in these circumstances. Fluorescence from both Hg and As peaks in this region overwhelmed the Br K_α at 11.92 keV. A small peak at 12.30 keV was attributed to both Pb $L_{\beta4}$ at 12.34 and Hg $L_\beta5$ at 12.27 keV. Pb $L_{\beta1}$ and $L_{\beta2}$ at 12.62 keV are not overlapped by other elemental lines but were intensified by the Fe K_α sum peak when the Fe concentration in the sample was high. The small Hg L_γ peak at 13.40 keV overlapped the Br and Rb peaks from untreated skin at 13.36 keV. Hg peaks at 13.83 keV and 14.21 keV were attributed Hg $L_{\gamma1}$, and Hg $L_{\gamma2}$, $L_{\gamma3}$, and $L_{\gamma6}$, respectively. Further peaks at 14.76, 15.16 and 15.75 keV are attributed to Pb L_γ emissions. A peak at 16.38 keV was attributed to a sum peak from Fe K_α and Hg L_α (6.40 + 9.98 keV). The final peak in this region at 16.93 keV was attributed to a sum peak of Fe K_α and As K_α (6.40 + 10.43 keV). Enhancement of Rh peaks at 20.17 and 22.70 keV were attributed to increased Rayleigh scatter.

In the higher energy region of the spectrum, numerous spurious peaks were identified and are listed below.

- 20.51 keV = Hg L_α + As K_α (or Pb L_α) sum peak (9.98 + 10.53 keV)
- 21.08 keV = Pb L_α sum peak (10.54 + 10.54 keV) which overlapped an As K_α sum peak at 21.06 keV (10.53 + 10.53 keV)
- 21.80 keV = Hg L_α + Hg L_β sum peak (9.98 + 11.82 keV)
- 22.35 keV = Pb L_α (or As K_α) + Hg L_β sum peak (10.54 + 11.82 keV)
- 22.60 keV = Hg L_α + Pb $L_{\beta1}$ sum peak (9.98 + 12.62 keV)
- 23.16 keV = Pb $L_{\alpha1}$ + Pb $L_{\beta1}$ sum peak (10.54 + 12.62 keV)
- 23.64 keV = Hg L_β sum peak (11.82 + 11.82 keV)

- 24.34 keV = Pb $L_{\beta 1}$ + As K_{β} sum peak (12.62 + 11.72 keV)
- 25.24 keV = Pb $L_{\beta 1}$ sum peak (12.62 + 12.62 keV) overlapped Sn 25.30 keV.

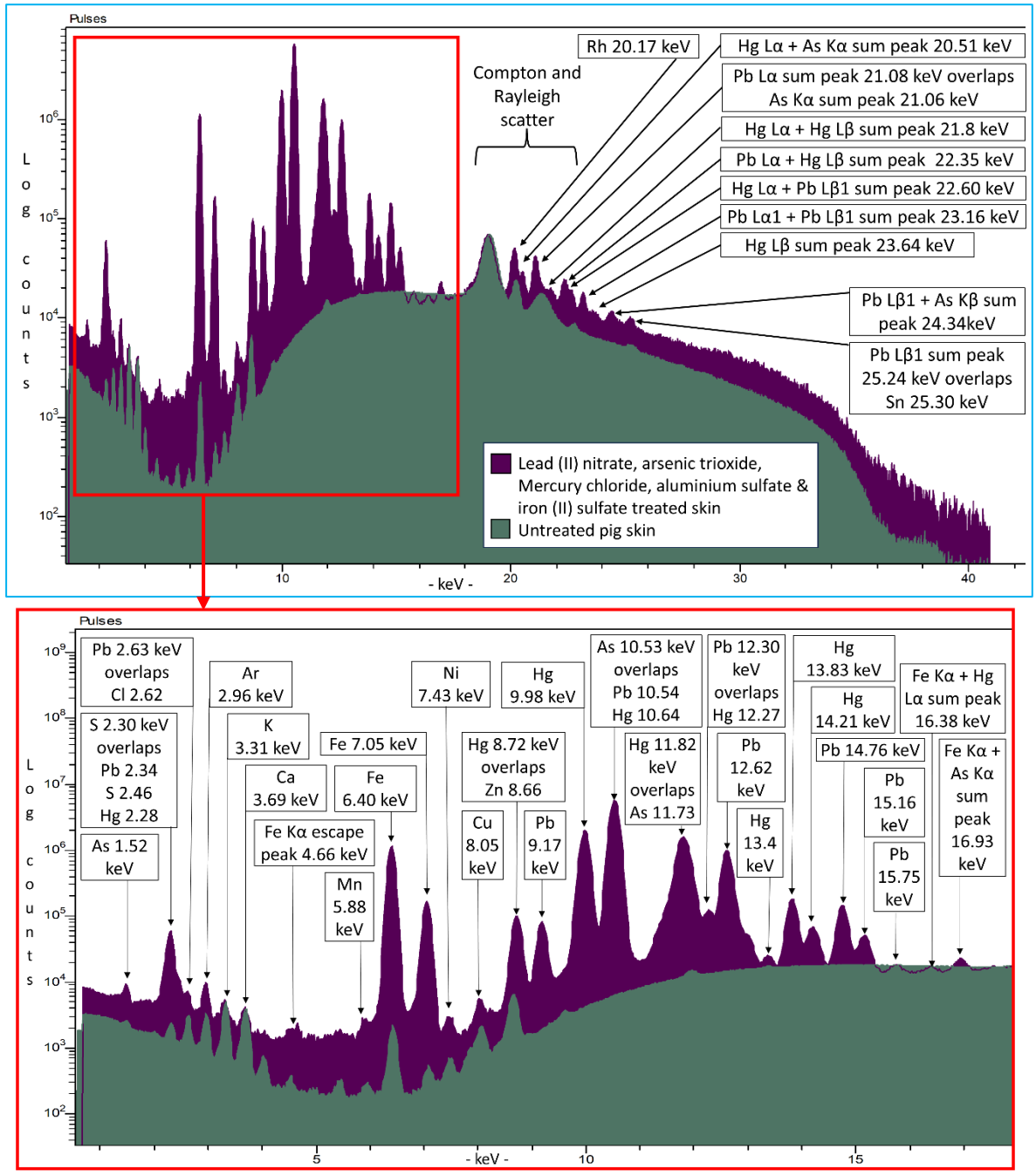

Fig. 2.17: XRF spectrum collected from three reference standards treated with lead(II) nitrate, arsenic trioxide, mercury chloride, aluminium sulfate and iron(II) sulfate, co-added using software accumulate function (dark purple), and overlaid with a spectrum collected from three untreated skin standards. Spectra have been normalised to the Compton peak.

2.4.3 Results – historic specimens

2.4.3.1 NHM457, rufous bettong (Aepyprymnus rufescens)

In Fig. 2.18, the XRF spectrum collected from the *Aepyprymnus rufescens* (rufous bettong) specimen NHM457 was dominated by Fe peaks at 6.40 and 7.05 keV (Fe K_α and Fe K_β respectively). This was supported by ICP-MS results, which showed Fe concentration in this specimen was 13 x 10^3 ppm. Intense spectral peaks were identified for Cl (2.62 and 2.82 keV) and S K_α (2.30 keV). The intense peak of K K_α at 3.31 keV suggested that this element was present in high concentration. The K K_β peak at 3.59 keV was overlapped by Ca K_α (3.69 keV) producing a broad peak.

Unlike the 1.48 keV peak in the pig skin reference standards, the peak in the data from NHM457 was quite clearly defined. In the pig skin standards, this peak (Al K_α peak at 1.48 keV) did not reflect the concentration of Al in the sample, and changes in the peak intensity were attributed to emissions from other elements including the As L_γ emission 1.52 keV. However, ICP-MS results established that the concentration of Al in NHM457 was 955 ppm, significantly higher than concentrations in the pig skin reference samples. As concentration in NHM457 was quite low, as evidenced by the As K_α emission at 10.53 keV. It is evident that the concentration of Al is NHM457 exceeded the detection limit of the instrument and therefore the peak at 1.48 keV reflects the presence Al in this sample.

A clearly defined peak at 1.74 keV was attributed to either Si (K_α) or Hg (M_2-N_1 transition) as both elements produce an emission at this energy. However, other characteristic peaks in the spectrum for Hg were not intense indicating that much of this 1.74 keV peak originated from Si in the sample. ICP-MS results confirm this, reporting 28.6 ppm of Si in this sample compared to Si concentration below the detection limit in the untreated pig skin standard.

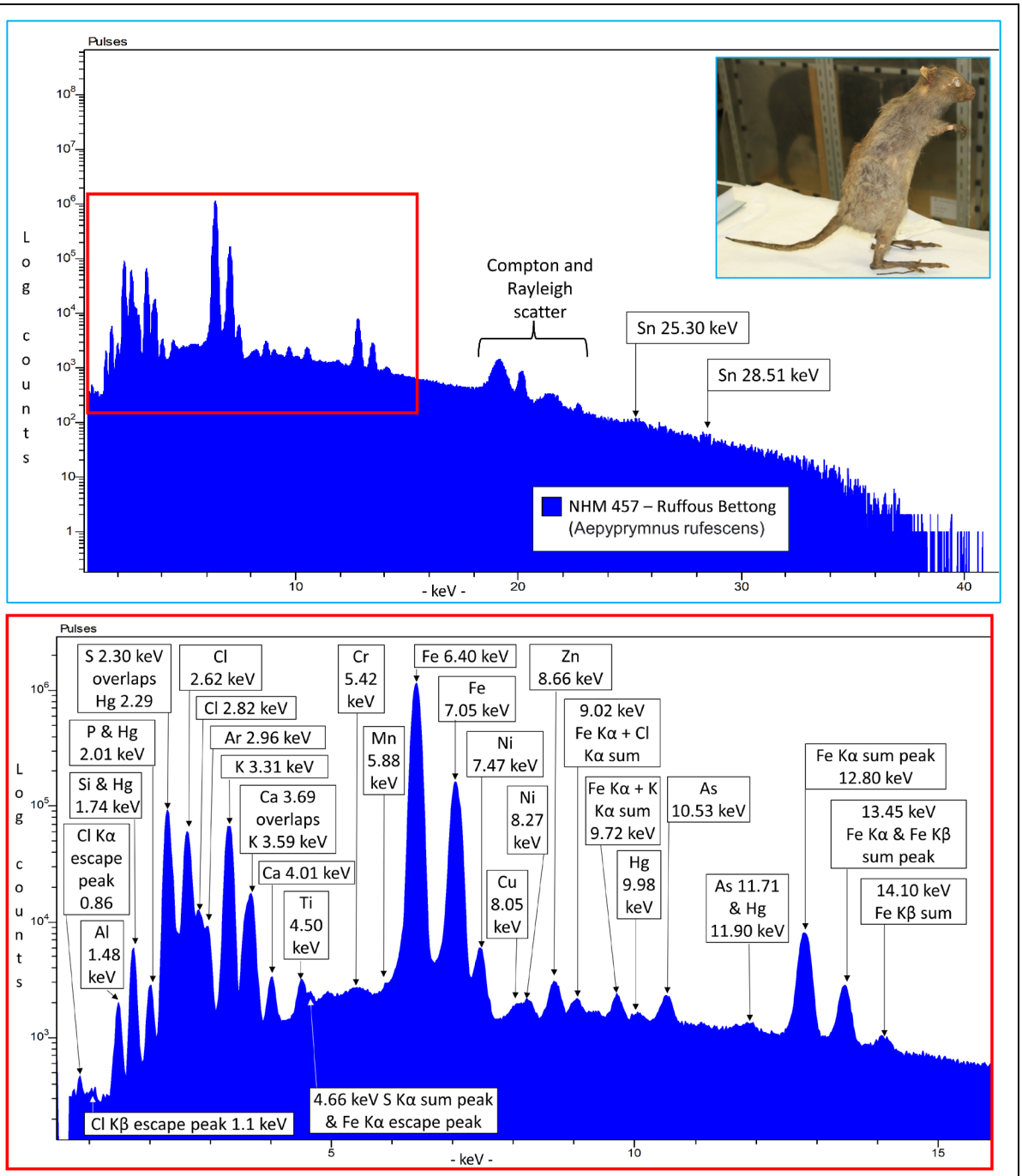

Fig. 2.18: XRF spectrum collected from a rufous bettong specimen NHM457 from the MAMU collection. Three spectra were co-added using software accumulate function.

A clearly defined Ni K_α peak at 7.47 keV coupled with a Ni K_β peak at 8.27 keV were also observed in the spectrum of NHM457. The literature does not suggest that Ni had a role as a preservative or disinfectant in the nineteenth century. However, Ni is an abundant element on earth, and its presence may be a result of contamination in one of the high concentration

preservatives applied. Ni commonly occurs as a natural alloy with Fe such as telluric iron and serpentine peridotites and in metallic meteorites (70). Interestingly, Si may also be present in naturally occurring Fe-Ni alloys e.g., suessite (71,72). Ni forms a salt with arsenic although ratio of the XRF peak intensities in this specimen does not support the nearly 50% Ni to As present in the naturally occurring alloy (73,74). In this case, it was postulated that the Ni and possibly Si in this specimen were associated with the application of an Fe based preservative (e.g., green vitriol) and that this may be useful in identifying the source preservatives applied to this specimen.

Numerous sum and escape peaks arising from each of the elements that had high intensity peaks, i.e., Fe, Ca, S, K were identified. These peaks are listed below.

- 0.86 keV = Cl K_α escape peak (2.26 - 1.74 keV)
- 1.10 keV = Cl K_β escape peak (2.82 - 1.74 keV)
- 4.66 keV = S K_α sum peak (2.30 + 2.30 keV)

  and/or Fe K_α escape peak (6.40 - 1.74 keV)
- 9.02 keV = Fe K_α + Cl K_α sum peak (6.40 + 2.62 keV)
- 9.71 keV = Fe K_α + K K_α sum peak (6.40 + 3.31 keV)
- 12.80 keV = Fe K_α sum peak (6.40 + 6.40 keV)
- 13.45 keV = Fe K_α + Fe K_β sum peak (6.40 + 7.05 keV)
- 14.17 keV = Fe K_β sum peak (7.05 + 7.05 keV)

With the elemental information provided through pXRF analysis, it was possible to reflect on the history of natural history preservation technologies with respect to NHM457. It was proposed that this specimen was prepared using a preservative recipe that included aluminium salts (alum), potassium salts (potash), and iron sulfate (green vitriol). These preservatives are consistent with practices of late eighteenth century recipe (75), and became less commonly

used in the nineteenth century when arsenic-based recipes gained popularity. This may indicate that the specimen was collected early in the 1800s, or that it was prepared at a time when the field collector's supplies were sufficiently diminished that they needed to draw on readily available local salts to make an historic preservative recipe.

2.4.3.2 NHB1354, red winged parrot (Aprosmictus erythropterus)

The XRF spectra collected from the museum specimen NHB1354, *Aprosmictus erythropterus*, is presented Fig. 2.19. Intense characteristic peaks for As (10.54, 11.73 and 1.28 keV), and Pb (2.30, 9.18, 10.53, 11.35, 12.28, 12.62, 13.01. 14.76, 15.14 and 15.76 keV) were identified. Peaks for Ca at 3.69 and 4.01 keV were also relatively intense. Hg peaks were identified at 1.74 (M_3-N_1 transition), 9.98 (L_α), 11.82 (L_β) and 14.26 keV (L_γ). The intensity of the S peaks at 2.30 and 2.46 keV were significantly enhanced by overlapping emission lines of both Pb (M_α) and Hg (M_β), which complicated interpretation of the importance of this element. Pb produced sum peaks at ~ 21.08 (L_α sum peak 10.55 + 10.55 keV) and around 23.16 keV (L_α + L_β sum peak 10.55 + 12.79 keV).

The elemental composition of NHB1354 was also considered in relation to the history of natural history preservation. The use of Hg was common for bird specimens throughout the period of Enlightenment (76). However, lead is not often mentioned in field collection manuals. Its presence in this specimen may point to the application of the pesticide lead arsenate which came into use the 1890s. For specimens with complex mixtures of preservatives and pesticides, such as this one, pure elemental composition can reveal limited information on a specimen's history.

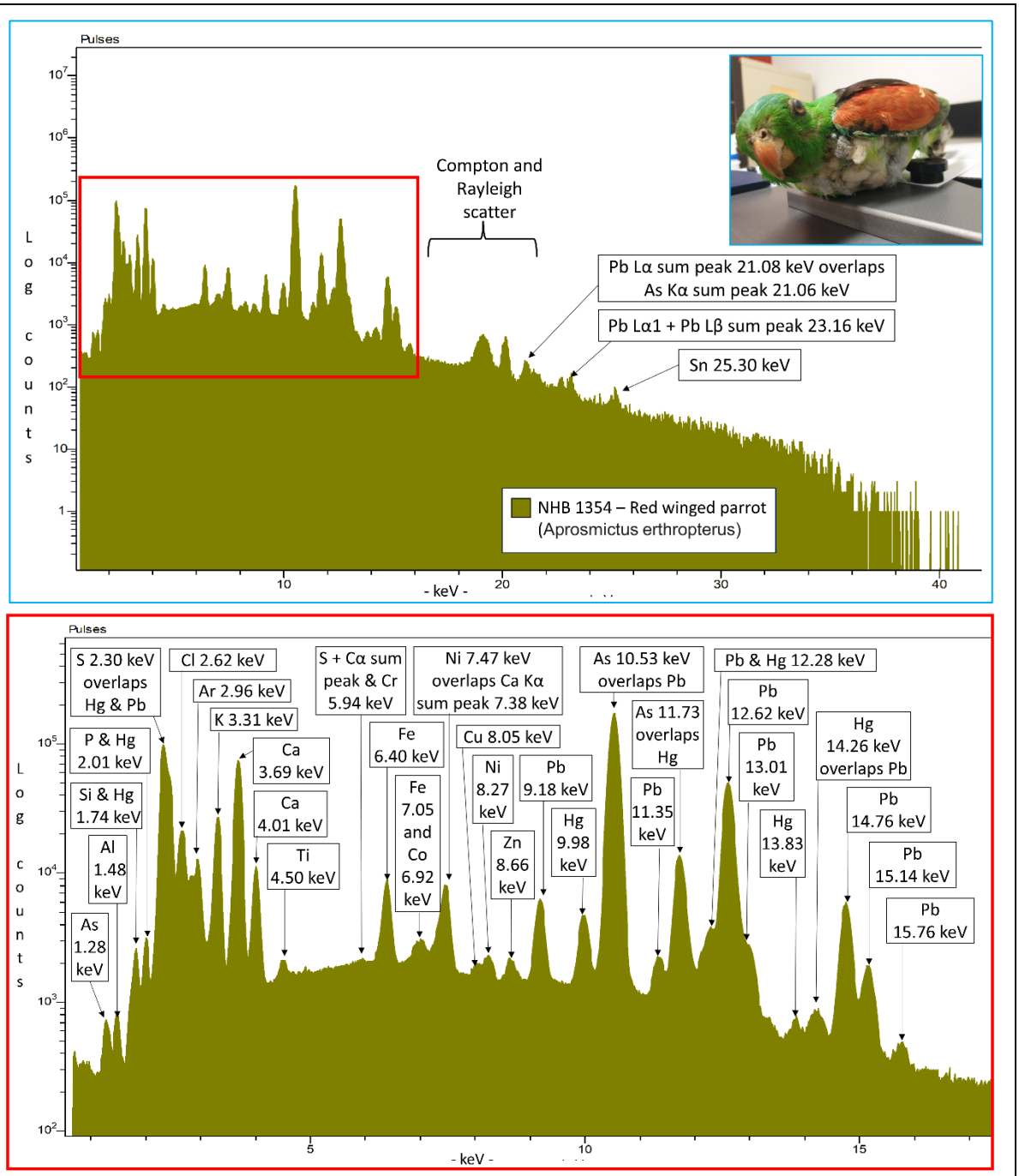

Fig 2.19: XRF spectrum collected from a red winged parrot specimen NHB1354 from MAMU collection. Three spectra were co-added using software accumulate function.

2.5 Conclusions

Through considering the theory of XRF spectroscopy and focusing on aspects relevant to the investigation of historic zoological specimens, an experimental protocol was developed which included procedures and mitigations that accounted for many of the expected matrix effects

associated with such complex material. The procedures were tested on reference samples treated with historic preservatives which revealed the interactions between X-rays, X-ray fluorescence, and a skin matrix.

Several ‘tips’ were identified as useful in the interpretation of XRF spectra produced from zoological material. The importance of recognising the contribution that the instrument itself makes to the spectrum was recognised. High concentrations of elements frequently produced sum peaks in XRF spectra collected from preserved skins, and this was the case for both high and low Z weight elements. Escape peaks, although less common, were also observed for elements in high concentration. However, Bragg scatter was not observed in these spectra suggesting that ground preservatives in a skin matrix do not often produce x-ray diffraction.

The detection limit for Al using this protocol is relatively high, consequently an absence of Al in a spectrum may not necessarily correlate with an absence of Al in the sample. The impact of the As Lα emission line must be considered when interpreting the Al peak (at 1.48 keV) in the spectrum. In specimens where arsenic, lead and mercury are present, both the As K_α (at 10.54 keV) and As K_β (at 11.73 keV) lines overlapped, thus the As L_α emission line (at 1.28 keV) may be helpful for identification. The Hg L_α peak (at 9.98 keV) may be used obtain a semi-quantitative understanding of Hg concentration in the sample without the influence from the common preservatives Pb and As. Pb L_ι peak (at 9.17 keV) or Pb $L_\beta 1$ (at 12.62 keV) offer a similar opportunity for semi-quantitative concentration of lead. Due to overlapping Pb emission lines, an understanding of the normal ratio of Pb peaks may be helpful in interpreting the importance of S (at 2.30 and 2.46 keV) in samples where Pb is present. The tail on the lower energy sides of both As K_α (at 10.54 keV) and As K_β (at 11.73 keV) may be misunderstood to represent the emission lines of elements including Thallium (ingredient in

rat poison). Therefore, when As is present in the sample, the identification of trace elements should be confirmed by using more than one emission line.

In applying the experimental protocol to samples collected from historic museum specimens, it was found that XRF analysis enabled the elemental composition of individual specimens to be compared to documented historic preservation practices. Subsequent conclusions could be drawn about the method of preservation and an approximate period of production could be proposed for one of the two specimens observed. The complicated mixtures of preservatives and pesticides limited further interpretation of elemental composition in the context of the history of natural history collecting technologies.

This Chapter has produced foundational reference material which was used throughout the subsequent chapters of this dissertation for the deconvolution and interpretation of XRF spectra collected from natural history specimens. However, this investigation showed that a more detailed analysis of XRF data is required to throw light on the historical significance of specimen elemental composition. The chemometric method, principal component analysis, has been demonstrated to successfully aid the interpretation of XRF data form historic objects, producing graphically represented results that can be readily interpreted (77-80). Chapter 4 explores the combination of principal component analysis with the XRF protocol documented in this chapter.

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Chapter 3

Dissecting Data Deficit

3.1 Introduction

The literature, described in Chapter 1, demonstrated the potential for a zoological specimen to provide physical evidence from which to reclaim information about its provenance. However, the analysis of two 'no data' specimens from the MAMU collection, in Chapter 2, showed that detection of the chemical residues remaining in historic zoological specimens is not sufficient to re-establish the detailed specimen data required for future research. It was proposed that a comparative study would yield more useful results - one in which specimens with no contextual data were compared with specimens of reliable provenance.

Qualitative comparative methodologies rely heavily on a framework of previously validated samples that may be used as a reference to compare new evidence against. Creation of such a framework for historic museum specimen is dependent on identifying reference specimens with reliable data. However, without a background understanding of the museum's history and its current data handling protocols, it is difficult to determine which specimens have authentic reliable data, and which have data with questionable validity. This chapter explores factors that have, and continue to, impact the veracity of the data attached to museum specimens. The purpose is to identify where specimens with reliable data may be found, and what types of data might best be approached with caution.

3.2 Dissociation, Atrophy and Obscuration of Contextual Data

The loss of contextual data may have been the result of a single catastrophic event (1). However, the extent of missing data, the variety of fields where deficits occur, and the dominance of this problem in older specimens, suggests that several of factors have contributed to the data deficits seen in museum collections today (2,3).

While the causes of data deficit may be diverse, the principal mechanisms by which contextual data are lost may be defined as:

- *disassociation*; where the contextual data and the specimen are no longer connected but both may still exist,
- *atrophy*; where the quality of contextual data is gradually and unintentionally degraded of over time; and
- *obscuration*; where some, or all, of the contextual data is intentionally removed or reinterpreted

The contextual data valued today may have had little purpose prior to the nineteenth century of natural history collection building (4,5). It is likely that specimen collectors at that time, required contextual information better suited to earlier understandings of nature (6,7). As the function and value of natural history collections changed, data needs gradually altered to fit the requirements of new classification systems and evolutionary concepts (8,9). However, until the late nineteenth century, knowledge of exactly which data were important to collect in the field was not well defined (10).

While field collecting was one of many ways a specimen entered a museum, each zoological specimen began its' "afterlife" (11) at the hands of a hunter or gatherer. In the field, what was important to the field collector was a significant determinant for the data collected, and this was heavily influenced by how the field collector believed the specimen would be used (2,12-14). In assessing data loss, one must consider that some contextual data may never have been recorded in the field. An example of such the lack of information about the collection locations of finch specimens captured by Charles Darwin in the Galapagos islands during the voyage of the HMS *Beagle* 1831-1836 (10,15,16). Before Darwin developed his theories on evolution,

and understood the importance of place, he overlooked the collection of location details for these specimens (15,16).

Fig. 3.1: Images showing the complexity of field collecting in nineteenth century Australia which may impact the quality of the specimen data. (**A**) Portrait of professional field collectors Robert Grant and Edward J Cairn on expedition in 1889 showing the sparse living conditions of a collector's camp (17). (**B**) In Australia, much of the work of field collection occurred within the context of the Frontier Wars represented in *'Bulla, Queensland'* 1861 by William Oswald Hodgkinson (1835-1900) (18). More information on the violet conflicts between Indigenous Australian sand colonists can be found at http://australianfrontierconflicts.com.au.

3.2.1 Challenging conditions

Even with specific instructions, the gathering of contextual data for each specimen was difficult. This was especially the case when expeditions combined exploration of new places with collecting natural history (19). Naturalists in the field often did not know their exact location and may have recorded their position relative to the last place travelled through (20,21). Accurate identification and naming of specimens also presented a problem, especially when unfamiliar animals were encountered (22,23). Taxonomy, then as now, was evolving and

the nomenclature of plants and animals was often modified (24,25), but few land-based collecting expeditions were equipped with extensive reference materials (26).

In addition to the practicalities of gathering data, collectors in the field were concerned for their safety, and unsure of their access to fresh water and sufficient supplies of food (26-28). They worked long hours to preserve specimens before the expedition moved on to new territory (29), and travelled laden with cumbersome equipment (30). Below John MacGillivray (1821-1867), describes the experience.

> "With the thermometer at 90° in the shade I had to carry water, ammunition, skinning materials, a double-barrelled gun, insect net, collecting boxes, a quantity of botanical paper and boards, besides two days' provisions. We steered our way by compass, and at night, after fixing our camp on a hilltop, had to keep watch against hostile natives" (31).

While on expedition, some naturalists exchanged money and goods for animals hunted by people unfamiliar with the need for contextual data (32,33). Others collaborated with Indigenous groups or individuals, drawing additional contextual data from their experience and knowledge while field collecting (34,35). In these cases, both the selection of animals/plants, and the quality and quantity of contextual data was influenced by the pathways taken through lands (36), by language differences (37), and by the choice of Indigenous naturalists to share their knowledge (38,39). It is perhaps wise to acknowledge that the nature-culture divide of the European scientific framework does not exist in many Indigenous societies, and the contextual data gathered in the field, may have far deeper cultural meanings. Thus, some gaps within existing contextual specimen data may be the result of the intentional obscuration by Indigenous peoples.

3.2.2 Data disassociation

The perilous journey from the field to a museum, specimen aggregator, or dealer, was the first of numerous events that eroded the contextual data successfully recorded in the field (40). In the nineteenth century, much of a specimen's contextual data was held in field journals or letters that were sent, separate from specimens themselves, to be reunited at the destination museum or collection (41,42). Specimens, journals, letters and labels (where present) may be disassociated from one another, lost, or destroyed on the journey (43,44). Alfred Wallace (1823-1913) recounts a story of specimen loss below.

> "…about 9 o'clock in the morning just after breakfast the Captain (who was the owner of the vessel) came into the cabin and said 'I'm afraid the ship's on fire. Come and see what you think of it' … My collections however were in the hold and were irrevocably lost. And now I began to think, that almost all the reward of my four years of privation and danger was lost." (45).

Upon arrival at the destination, the risk of data and specimen becoming disassociated continued. Although "every possible care..." (46) was taken, Customs officials inspected specimens for assessment of import duties and sometimes muddling the labels and documents (41,43) resulting in the loss of critical information. Specimens that were traded between museums and collections ran this gauntlet each time they changed ownership (47). Lucas and Lucas have shown that contextual data deterioration was directly related to the number of custodians used to facilitate trades and transactions (48).

Across the nineteenth century and around the world, public museums were being created, each demanded the rapid supply of specimens to represent the diversity of life on the planet (49). Upon arrival, newly acquired specimens required marrying to field records, and transcribing into the museum register or catalogue (33). The large volume of incoming specimens from expeditions, and new acquisitions through trade and purchase, increased the chance of errors

in cataloguing and transcription (22,50). The Philosophical Society of Victoria's Murray River Expedition in 1857, for example, produced 16,000 specimens to be registered on their arrival at the museum (51). The time required to register acquisitions meant that this work may be delayed, increasing the chance of error while simultaneously reducing the possibility to verify details with the expedition team or specimen trader (42). In remote museums, the curator and field collector were one and the same. Thus, an exhausted field collector would begin the curation of new and traded specimens on their return to the museum (8).

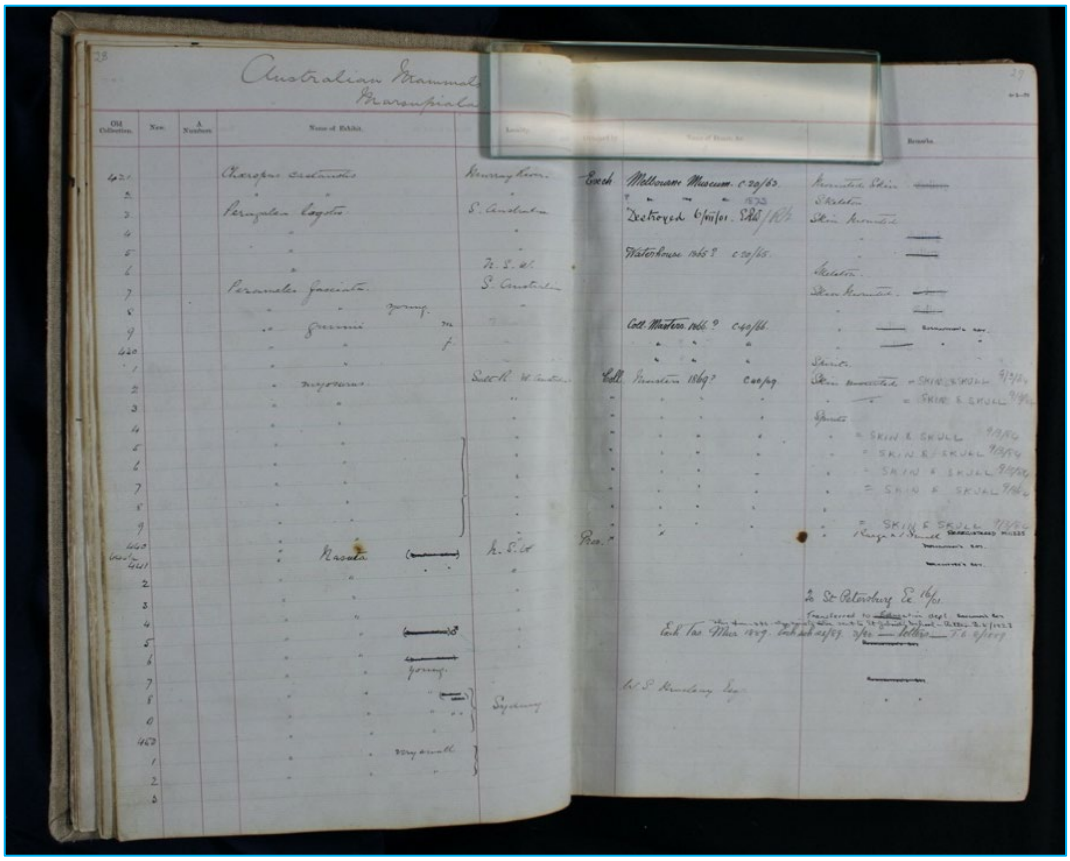


Fig. 3.2. In the Australian Museum's Palmer Register, the possible fields for specimen data were "number", "name of exhibit", "locality", "obtained by", "name of donor", "remarks" notes. The absence of a column for date, and the assumption of "donor" suggests this register served as an accounting document rather than a record of information about the animal itself. Image produced with permission form the Australian Museum (52).

3.2.3 Data atrophy

The space for each entry in the early museum registers and catalogues was typically limited, allowing for only the simplest information to be recorded (53). Information considered worth preserving was transferred to registers, labels, and reports while information considered useless was omitted. Curatorial practices of the nineteenth century included the removal of legacy labels from incoming acquisitions and transcribing only useful information to new labels (33,54). In the Australian context, Aboriginal Knowledge recorded in the field, for example, did not align with the knowledge systems of nineteenth century zoology, and were typically not transcribed into museum catalogues (39). Thus, a cycle of data evaluation and re-evaluation commenced and with it, a gradual atrophy of the originally recorded contextual data (55).

Over time, specimens were traded, relocated, stored, reconfigured, and displayed often requiring changes in the catalogue and on specimen labels (42,50). Some nineteenth century curators were better at handling data than others (56). Errors made in transcription of data; confusion from different styles of writing dates; and discrepancies between catalogue and labels; difficulties in reading handwritten, faded, damaged labels; and translation from foreign languages all contributed to data atrophy (57-60). Collector’s field journals may have been overlooked or were unavailable, which inhibited the transcription of contextual data into the museum catalogue (42). William Blandowski, for example, left Australia with field records for all the specimens submitted to the Victorian University Museum after the Philosophical Society of Victoria's Murray River Expedition (1857), taking the contextual data with him (23). Catalogues were updated to modern databases, and these then upgraded, each time requiring a re-evaluation and transcription of each specimen’s existing contextual data into the scientific categories and physical spaces of new data storage systems.

3.2.4 Data obscuration

Amid the excitement of the nineteenth century amassing and trading natural history collections were instances of intentional data obscuration and the creation of fraudulent data. Celebrated ornithological collector, Richard Meinertzhagen, notoriously stole specimens from other collectors and collecting institutions, reworking and relabelling them, and in some cases adding false contextual data (61-63). Specimens supplied by field collector, Henry H Travers, have been the subject of much research in recent decades, which has revealed numerous specimens with false collection dates and locations (35,64). Professor Joseph H Batty, author of nineteenth century field collecting and taxidermy manuals, was revealed to have sold numerous specimens with false field collection locations and dates (33,65). Specimens associated with Australian field collector, John Thomas Cockerell, are shrouded in doubt as numerous examples have been discovered where the collection location has been fabricated (66). Debate still continues on the, so-called, 'Hastings Rarities' in which taxidermist George Bristow was accused of falsifying location data for over 595 specimens to secure higher sale prices (21). Gerard Krefft reported, with some amusement, that the Nyeri Nyeri collectors, who aided him in the Australian Philosophical Society of Victoria's Murray River Expedition (1857), attempted to sell skins of the bandicoot *Perameles obesula* with the tail removed in place of the rarer tail-less bandicoot, *Choerupus ecaudatus* (51). The relationship between rarity and high pecuniary value may have been a significant driver in the falsification of contextual data (33,35).

Alongside operational mechanisms of data atrophy, museums were evolving from assemblages demonstrating the diversity in nature, to institutions claiming the creation of natural knowledge and 'science' (67,68). Natural history too, was evolving, as new zoological specimens, flowing into museums from field collecting expeditions necessitated reinterpretations of the natural world (2,39). These changes influenced the use, evaluation, and retention of contextual data

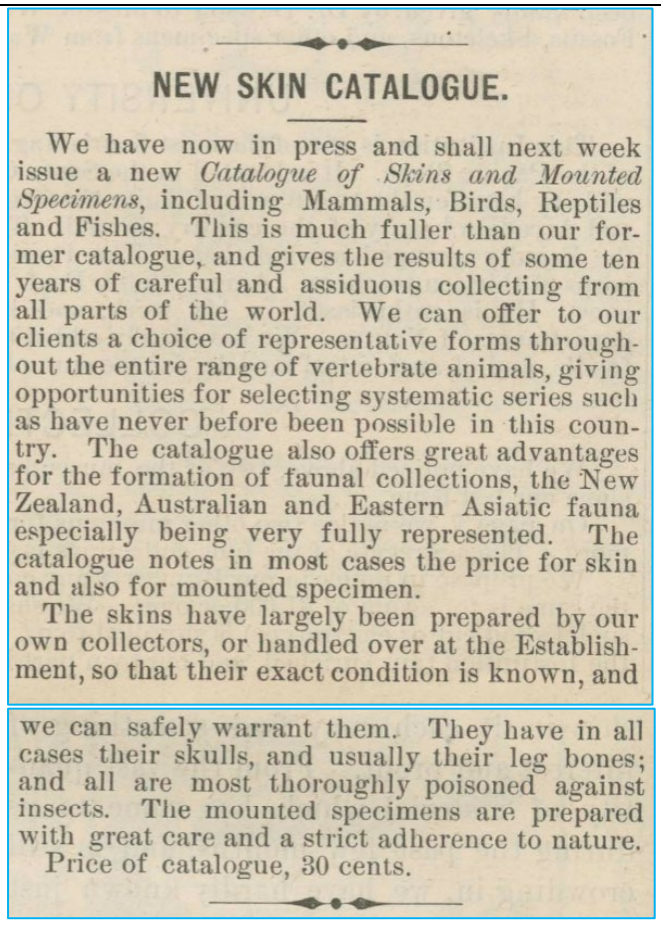
(69,70). In zoology, the widespread absence and ambiguity of contextual data for the field collector is an interesting example.

3.2.5 Data deficits and field collector information

Nineteenth century natural history field collecting required a very specific combination of skills and knowledge. Firstly, the ability to recognise objects that may be significant or 'new to science' and to select animals that may serve as representatives within existing frameworks of scientific understanding and record relevant contextual information. This had a profound impact on every other interpretive act of knowledge-making that followed (71). Second, the skill to capture the biological material with which to make a specimen without significant damage. Third, the knowledge and skills required to prepare, preserve, and pack specimens for transport from the 'wild' and into 'society'. As we have seen above, field collecting can be a collaborative action between two or more skilled people.

The skills and experience of a field collector was an important factor in the quality, and durability of the specimens created, and was foundational to the success of professional field collectors (67,72,73). Specimen quality also impacted the reputations of the dealers, and museum curators involved in specimen trades (43). This is demonstrated in the efforts made by museums to hire their own experienced collectors and taxidermists (74), and by the careful description provided in Ward's Natural Science Bulletin (75) (Fig 3.3). Curator of the South Australian Museum, F. G. Waterhouse regarded specimens collected by Andrews and Schultz as particularly valuable in trades with other museums (66). As such, it is likely that the field collectors' identity was made available to specimen aggregators, museums and purchasers. Today too, it is acknowledged that the initial preservatives applied in the field, have the "greatest influence on a specimen's condition" (76). Yet in modern specimen data, the near "anonymity" (77) of the individuals that made such critical decisions is remarkable.

Confusion over the term ‘collector’, meaning both field collector/ hunter and a person who aggregates specimens into an assemblage, caused its own type of atrophy for data that related to field collection (68,78). Not all museum catalogues, however, had a space reserved for both field collector and specimen supplier. This necessitated a decision about which of these two types of ‘collector’ data would be retained. Catalogue entries often had the name of the exchange partner, dealer, or donor in place of field collector, particularly for specimens that were traded, exchanged, or donated into museum collections, (43,69). However, this loss of data was more than simple misinterpretation. Ensuring equitable negotiation of exchanges, donations, and purchases within a broad network of trading partners required a record of the supplier for each specimen.



NEW SKIN CATALOGUE.

We have now in press and shall next week issue a new *Catalogue of Skins and Mounted Specimens*, including Mammals, Birds, Reptiles and Fishes. This is much fuller than our former catalogue, and gives the results of some ten years of careful and assiduous collecting from all parts of the world. We can offer to our clients a choice of representative forms throughout the entire range of vertebrate animals, giving opportunities for selecting systematic series such as have never before been possible in this country. The catalogue also offers great advantages for the formation of faunal collections, the New Zealand, Australian and Eastern Asiatic fauna especially being very fully represented. The catalogue notes in most cases the price for skin and also for mounted specimen.

The skins have largely been prepared by our own collectors, or handled over at the Establishment, so that their exact condition is known, and we can safely warrant them. They have in all cases their skulls, and usually their leg bones; and all are most thoroughly poisoned against insects. The mounted specimens are prepared with great care and a strict adherence to nature.

Price of catalogue, 30 cents.

Fig. 3.3: 1882 advertisement from Ward’s Natural Science Establishment show that skilled specimen preservation was important to their clientele (75).

At the same time, objectivity became increasingly important in scientific knowledge making, and the human intervention that made an animal into a specimen contaminated that objectivity (2,6,70,79). Conversely, the connection between a specimen and the perceived scientific objectivity possessed by unpaid 'gentlemen' naturalists, was thought to support the authenticity of both specimen and data (72). Association with a geographically fixed supplier (as opposed to an explorer, traveller, or professional field collector) simultaneously disguised the geographical inaccuracies inherent in nineteenth century mobile collecting practices, and lent credibility to location data (53). But the obscuration of field collector data may have been a more personal decision. Intertwined with the movement of specimens and their contextual data, another type of exchange was taking place, that of reputation and scientific recognition (80,81). In exchange for the supply of new-to-science specimens, natural scientists described animals with an eponym of the collector/supplier (53,82,83). The field collector's name may have been substituted with the name of expedition leaders, exchange partners, donors, or ambitious curators ensuring that they may be credited in any subsequent descriptions (84).

3.2.6 Modern data atrophy and issues of data reconstruction

Natural History Museums continue to evolve today in response to shifts in community understandings of past social and natural environments (69,85). Specimen contextual data continues to be reinterpreted and re-evaluated, and access updated to facilitate the needs of collection users (55,86). While the Darwin Core helps standardise many of the terms used in recording specimen data (57,87), the discrimination between original contextual data, and reconstructed data, is handled differently by each organisation. This complicates recognition of compromised data within the vast number of natural history specimens in global data sources. Older specimens, with deficient contextual data do not fit neatly into modern data structures (60,88).

Grappling with modern collection management databases has many parallels with the paper catalogues of the past. Pre-defined data fields support discovery and retrieval of existing data but demand the stripping away of details and nuances within the contextual data. The recent retrospective geocoding of historic location data has created its own wave of data atrophy (60). Identification of collection locations in early specimens is hampered by changes in national boarders, place names, and the historical use of same name applied to more than one location (33,89,90). General place names provided in the historic contextual data have been replaced with equally generalised geocodes (88,90) without the nuances of the written description, e.g., "near coast".

3.3 Discussion and Conclusion

As discussed in Chapter 2, selecting study skin specimens of a limited number of species and from a single museum was considered optimal for limiting aspects of inter-specimen variability. The Australian Museum was approached to provide access to specimens for a pilot study. Formal applications and informal discussions led to the acceptance of the analytical XRF protocol described in Chapter 2 and agreement on mitigations required to limit the risk of damage to specimens. The optimal size of the animals was then determined in collaboration with museum staff. This decision was based the size of the workspace at the museum, instrument size, and the requirement for several discrete sites of measurement for each animal to establish chemical composition and distribution. It was agreed that specimens would be limited to a length range $\geq$ 20cm and $\leq$ 80cm.

The investigation into the nature of museum data and that causes of deficits informed the subsequent selection of specimens. Based on the understanding that traded specimens were more likely to have lost their data, it was decided that Australian animals were least likely to be affected by data loss related to transfer of ownership. This same reasoning directed specimen selection toward those collected specifically for the Museum. The Australian Museum has

employed a number of field collectors over the nearly 200 years of its operation. It was recognised that the specimen acquisition lists held in the archives and published in the Museum's annual reports could be used as a supporting resource, if questions of data validity arose.

The Museums digital catalogue was accessed with these objectives in mind. The collectors Kendall Broadbent (1837-1911), Robert Grant (c.1854-1923), John A. Thorpe (unknown-1907), Henry Sneddon Grant (c.1892-1944), and George Masters (1837-1912) were each employed by the Australian Museum as collectors during the nineteenth century. Of these, only study skins collected by Broadbent, Masters, R. Grant and H.S Grant had sufficient duplicates, of appropriate size, to form a robust sample group. A group of twelve Pademelons (*Thylogale* spp.) collected by Broadbent and by R. Grant were selected to form a pilot sample group.

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Chapter 4

Pursing Pademelon Provenance

Foreword

The hypothesis that chemical residues may be characteristic of individual field collection practices of nineteenth century field collectors was presented in Chapter 1. Chapter 2 described the protocol of data collection from historic specimens but demonstrated that without further analysis, the XRF data has limited potential in identifying relationships between chemical residues and historic field collectors. Chapter 3 presented the decision to use a comparative analysis to further investigate specimen provenance and explored the importance of understanding the validity of specimen data used for establishing a framework of 'known' values.

The findings from the previous chapters are brought together here, in Chapter 4. For the first time, carefully selected specimens with reliable contextual data were analysed using the established XRF protocol and combined with principal component analysis (PCA).

This chapter was published in Heritage Science.

Cramer, C., Carter, E.A., Swarbrick, B. Philp, J, Pay, P.A. Pursuing pademelon provenance: a pilot study using portable XRF to trace field-collection of museum mammal specimens. *Herit Sci* 2023 25 July; 11:151. https://doi.org/10.1186/s40494-023-00998-1

Cramer *et al. Heritage Science* (2023) 11:151
https://doi.org/10.1186/s40494-023-00998-1

Heritage Science

RESEARCH Open Access

Pursuing pademelon provenance: a pilot study using portable XRF to trace field collection of museum mammal specimens

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Abstract

Internationally, the value and usefulness of museum zoological specimens are compromised when supporting contextual data are lost or disconnected from the specimen. In this pilot study, twelve *Macropodidae Thylogale* (pademelon) skins with known provenance from the Australian Museum (Sydney) were analysed using portable X-ray fluorescence spectroscopy and principal component analysis. Elemental composition of preservative residues was assessed to establish if common patterns existed and could be associated with particular field collectors. Specimens were differentiated, and the field collector deduced, based on elemental analysis of preservative residues on skins. Each of the nineteenth century field collectors, in this study, were found to have applied the same or similar preservatives to zoological specimens over a number of years, which showed a consistent pattern of practice. Additionally, the specimens obtained by each of the field collectors could be distinguished from one another based on the preservative residues. These discoveries provide exciting prospects for the use of X-ray fluorescence spectroscopy to couple museum specimens with unknown contextual data via their field collector and associated archival evidence, and hence, enable a considerable enhancement of their value as museum and research objects.

Keywords Natural history museum, Portable X-ray fluorescence spectroscopy, Provenance, Zoological specimens, Taxidermy, Principal component analysis

4.1 Introduction

During the nineteenth century, innumerable mammal skins were collected, traded, and incorporated into natural history collections around the world [1–5]. Each skin was typically associated with information about the context from which the animal was removed. Such data, including geographic location and collection date, are essential to enable global research into biodiversity, systematics, health, and historical studies [6–14]. Through the cycles of exhibition and changes in data storage mechanisms, many older specimens have become disassociated from their contextual data [15]. Without this critical data, specimens are left "historically unreliable" [16] and regardless of their rarity or unique characteristics, these specimens have a greatly diminished value in furthering scientific research [17–19].

Zoological specimens with missing data are widely acknowledged as a problem when using natural history museum collections in research [20–22]. Several studies have attempted to quantify the extent of this problem with the use of publicly available biological collection data aggregators, but the results are often interwoven with database filtering and retrieval complexities [23, 24]. However, a handful of surveys have documented the number of records found that have a complete absence

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of data for specific fields, and these may provide a rough indication of the number of specimens that are affected by data loss. Using the Global Biodiversity Information Facility (GBIF), Gaiji, et al. found 26.8% of specimens globally were without data for year of collection [25]. Malaney and Cook, using Vertnet, found 6% of mammal specimens in USA museums were missing a year of collection [18]. Peterson, et al., with a selected data set from Vertnet, found 4.6% of ornithological specimens had no location information [26], and the Australian Environmental Resources Network reported 18% of specimens in Australian collections had erroneous or missing location data [27]. Based on these studies and using the estimate of 2.1 billion specimens in the world's museums [28], 6% represents as many as 120 million specimens that cannot be used in research where time is an important factor because of missing date data.

The 'Merchants and Museums' project investigated pathways to reconnect 'no data' museum specimens with archival and historical data [29]. Within this project, it was proposed that the chemical residues from preservatives used on zoological specimens may lead to the identification of the field collector and, thereby, provide the necessary viable links to archival and other contextual data to confirm or re-establish provenance.

Numerous analytical methods have been applied to zoological museum specimens, contributing to individual object histories by determining pesticide and preservative use, including Fourier transform infrared spectroscopy [30, 31], inductively coupled plasma mass spectrometry [32, 33], and atomic absorption spectroscopy [5, 34]. Mass spectrometry and subsequent stable isotope analysis has been successfully applied to determination of the geographic origin of zoological material by focussing on chemical residues acquired by an animal during life [35–37]. The biggest limitation to the use of these analytical techniques is the necessity to remove a sample from the specimen, and often destruction of the sample via the analysis. Such destructive sampling progressively diminishes the irreplaceable zoological resource that museums provide [38, 39]. In contrast, non-invasive spectroscopic analytical techniques enable the characterisation of zoological specimens without physical sampling thus supporting museums' aim of in-perpetuity preservation for future research and education [40].

The use of portable X-ray fluorescence (pXRF) spectroscopy is well established in museum practice due to its capacity to yield compositional data on multiple elements at the same time, without physical sampling, or the relocation of heritage objects to an external site for analysis, at relatively low cost [41]. In air, it is sensitive to elements with atomic numbers above magnesium and provides immediate elemental information in the form of counts (of fluorescent X-ray photons), that are linearly dependent on the concentration of each element in the sample [42, 43]. It is particularly well suited to comparative studies [44] and its application has successfully revealed histories of manufacture, and the provenance of inorganic materials used in historic objects [45–48]. However, the technique has been underused in the study of museum zoological skins for exploring historical methods of specimen manufacture and has not previously been successful in establishing provenance including the 'when' and 'where' a live animal was made into a zoological specimen. In museum specimens, pXRF has been used almost exclusively for qualitative and semi-quantitative measurement of elements toxic to humans for the purpose of managing the exposure of workers and visitors to poisonous chemicals [31, 49–52]. This limited use may be attributed to the difficulty in applying proven pXRF methodologies for establishing provenance of inorganic materials, which compare elemental concentrations in parts per million, to biological material [49, 53–56]. Obtaining accurate quantitative pXRF data from biological material is complicated by inherently high concentration of low atomic number elements which cannot be detected by pXRF in normal atmosphere, as well as heterogeneity in skin porosity and density, particle size, surface geometry, and absorption or enhancement interactions between elements [57–63]. For zoological museum specimens, this is further complicated by variations in the distribution of hand applied preservatives, and in the difficulty in separating preservative residues applied in the field, from undocumented pesticides applied via routine treatments in the museum [64–66]. Thus, the full potential of pXRF to aid research on the histories and provenance of museum zoological specimens is yet to be realised.

Over 250 instruction manuals and pamphlets were published during the eighteenth and nineteenth centuries each including at least one preservative recipe [67–69]. By the mid nineteenth century, instruction manuals offered several preservative mixtures for each zoological class indicating that practitioners selected the recipe that best suited their situation. However, it was not always clear which mixture should have been or was used under which circumstances. Changes in the nomenclature of ingredients added further ambiguity to our interpretation [67, 70]. Literature on the history of specimen preservation has inferred that nineteenth century field collectors followed the trends and innovations documented in these contemporary instruction manuals [71, 72]. In contrast, Merle Patchett's and Adrian van Allen's work on the craft nature of specimen preparation, hypothesised that specimen preparators were less influenced by innovations and

trends, but were likely to have maintained the unique actions passed down from their teachers [73–76]. However, field records documenting the preservative mixtures collectors applied in the field, are rare both in Australia and the rest of the world [34, 77–79]. It is not clear if field collectors always used the same recipe over several years, used different preservative mixtures for specific climatic conditions, or updated their practices in response to published innovations. For field preservation, neither position has been supported by archival field records nor by physical evidence from the specimens themselves.

In this proof-of-concept investigation, a zoological specimen is recognised as a man-made object, created with the application of preservatives at the time of the animal's death [73, 75]. By focusing on the residues applied at this moment, this study explores that capability of pXRF spectroscopy to provide evidence of nineteenth century field collection practices, and to determine when and where a zoological specimen was extracted from the wild. To achieve this, analytical methodologies previously applied to inorganic materials in artistic and archaeological objects, and in geological science [45, 53, 56, 80, 81], were adapted to the semi-quantitative nature of XRF data collected from biological material [55].

Principal component analysis (PCA) was used to investigate the similarities and differences amongst the specimens. This multivariate statistical analysis method addresses trace and bulk elements alike and simultaneously, allowing for the identification of difference between specimens regardless of concentration of those elements. PCA extracts the latent relationships within and between data sets and condenses the most important information in the data into a series of principal components (PCs). PCs are ordered and numbered based on the quantity of information within the data set that they describe, with PC1 describing the most information and subsequent PCs describing less. The information held within each PCs is presented as a Loadings plot. Data from individual pXRF measurements are plotted as a point within a Scores plot. Points that cluster together are similar, and separation between clusters indicate dissimilarity. Scores and Loadings plots are interpreted together to describe the similarities and differences between specimens [82, 83].

The aim of this pilot study was three-fold:

1. To test the capacity of the experimental protocol to identify and/or differentiate between individual specimens
2. To determine if collectors followed a consistent pattern of practice over different expeditions that may be used to produce a characteristic elemental profile or fingerprint; and
3. To investigate if specimens acquired by two or more collectors can be differentiated using these elemental profiles.

4.2 Experimental

4.2.1 Portable XRF spectroscopy

Analysis was performed using a Bruker Tracer 5i portable XRF spectrometer (Karlsruhe, Germany; heretofore referred to as Tracer), equipped with a rhodium (Rh) thin-window X-ray tube; X-ray generator 6–50 kV with 4.5–195 μA, maximum 4 W output) and a 10 mm^2 silicon drift detector (Proprietary 20 mm^2 silicon drift detector with 140 eV @ 250,000 cps Mn Kα; resolution for optimum light element analysis.). Specimens were analysed in air at 40 kV and 30 μA for 40 s with a Ti 25 Al 300 filter and using an 8 mm collimator.

XRF spectra were collected in situ at the Australian Museum in accordance with the conditions defined in the NSW Radiation Control Act 1990 [84], and by a certified portable pXRF operator. The Tracer was mounted on its stand beneath a custom-built specimen stage that enabled the pademelon skin to rest, supported, above the nose of the instrument. No preparation was undertaken prior to analysis of the skins. Skins were positioned so that a relatively flat surface was presented to the nose of the instrument. Fresh nitrile gloves were stuffed with kitchen paper and manipulated into shape to support the specimen in position and prevent movement during spectral collection. The instrument was controlled via a laptop using Bruker ARTAX software (v8.0.0.476).

Appropriate protocols were followed to ensure the safety of researchers and museum staff including the use of required personal protection equipment when handling specimens. Analysis was conducted in a well-ventilated room with restricted access to visitors. Specimens were handled by trained museum object handlers. Gloves were replaced between each specimen to minimise the risk of cross-contamination.

A minimum of twenty sites were analysed for each *Thylogale* in order to provide data that represented the whole object, and to account for variations in skin and preservative application, as well as cross-contamination and pesticide exposure from within the museum environment. A minimum of two spectra were collected from the belly, back, left and right sides, and the head. Additionally, all specimens had at least one area where no fur remained on the skin, typically where the legs rested against the shelf in storage. At least one spectrum was collected from the bald area for each specimen. The specimen stage was cleaned with 70% ethanol and water, and disposable

paper towel after each specimen then fresh supports were assembled.

4.2.2 **Data analysis**

Spectra with dead times above 10%, or low signal, were excluded as they were considered unsuitable for reliable elemental analysis. ARTAX software (v7.8.2.0 Bruker) was used to conduct Bayesian deconvolution on the remaining 218 XRF spectra, with escape and pileup peak, and background correction enabled. Peak area data were then normalised to the Compton peak area (18.5-19.5 keV) of each spectrum in Microsoft© Excel. No further pre-processing was undertaken. Resulting data were imported into VEKTOR DIREKTOR (v1.1 KAX Group, Australia) and principal component analysis (PCA) was performed using random cross-validation, and mean centring for 1000 iterations over five components.

4.2.3 **Materials**

Unlike taxidermy mounted specimens, research study skins typically undergo few preservation treatments after their arrival at a museum [85]. This i nvestigation a nalysed only study skins to minimise the potentially confounding effect of chemicals and contaminates arising from subsequent preservation and pesticide treatment to the skin. To further reduce possible confounds from the museum's environment, specimens were selected from a single museum, The Australian Museum. Specimens were selected from a single genus to maintain comparable thickness and density of the skin and fur, and to reduce possible impact of endogenous elemental residues associated with significantly different animal diets.

In many cases, a museum will have only a few representative specimens of each species making the selection of a large group of specimens challenging [59, 78]. However, The Australian Museum holds over thirty-five *Thylogale (Marsupialia: Macropodidae,* known as pademelon) study skins (Fig. 1). Twenty-one of these are attributed to professional museum collectors, Kendall Broadbent (1837–1911), Edwin James Cairn (unknown-1939) and Robert Grant (c.1854–1923). From this group, three species from three distinct regions were chosen to assess the impact of the animal's habitat on elemental differences and specifically on preservative choice. The coastal New South Wales (NSW) habitat of *T.thetis* is typified by temperate dry forests; the Far Northern Queensland habitat of *T.stigmatica* is characterised by tropical rain forests; and the climate in central Tasmania, the habitat of T.*billardierii,* may be described as cool temperate forests and grasslands. The field notes held at the Australian Museum archives for all three of these collectors did not include information on the preservatives applied in the field, and no further documentary evidence on their practices was identified at the outset of this study.

A total of twelve *Thylogale* study skins representing three species (*T.thetis*, *T.stigmatica*, and T.*billardierii*) were selected (Table 1). Six pademelon skins represented two collecting expeditions by Kendall Broadbent: Two T.*billardierii* were collected during an 1897 expedition to Tasmania [86] and four *T.stigmatica* were collected during an 1880 expedition to Queensland [87]. Three *T.stigmatica* skins represented two collecting expeditions by Robert Grant working with E.J. Cairn: an 1887 expedition to the Bellenden Ker Ranges [88] and an 1889 expedition to Herberton [89] both in Northern Queensland. Three *T.thetis* skins represented one collecting expedition undertaken by Grant alone: an 1892 expedition to Seal Rocks and the Bellinger River NSW [90]. All the specimens selected were stable, with no significant

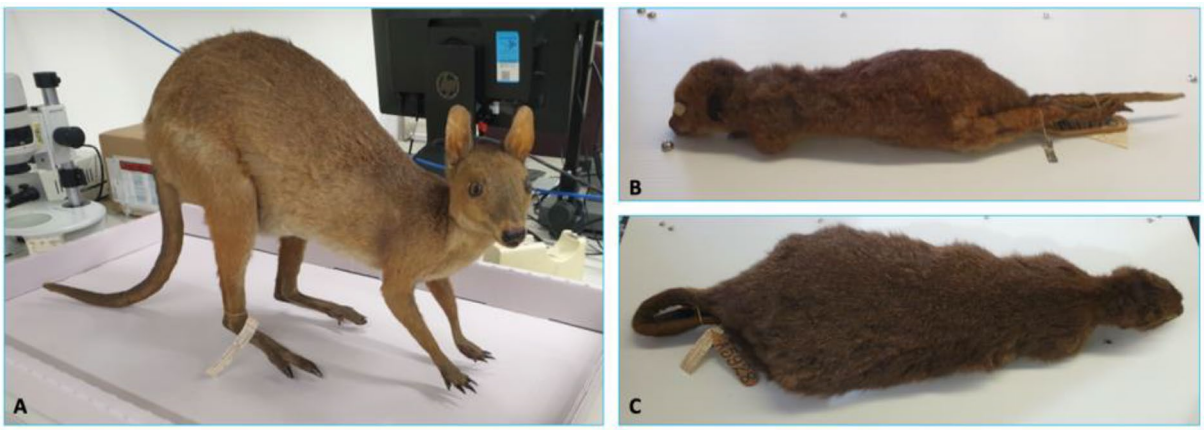

Fig. 4.1 Examples of *Thylogale* specimens at the Australian Museum. **A** *Thylogale stigmatica* adult mounted skin A. 1370 **B** *Thylogale stigmatica* juvenile study skin M.128. **C** *Thylogale billardierii* adult study skin A. 5928

Table 4.1 *Thylogale* study skins included in this study

Field collector	Expedition year	*Thylogale* species	Collection location	Accession number
Broadbent	1879	*billardierii*	Tasmania	A5339 A5928
Broadbent	1880	*thetis*	Darling Downs, QLD	A9721 A9722 A9725 A9726
Grant and Cairn	1888	*stigmatica*	Bellenden Ker, QLD	M128 M129
Grant and Cairn[a]	1889	*stigmatica*	Herberton district, QLD	M494
Grant	1892	*thetis*	Bellinger river, NSW	M793 M796 M798

[a] Although registered as collected by Grant and Cairn, the report from the collecting trip shows Grant was not with the expedition when Cairn collected M494 [91]

differences in skin or fur condition to suggest any differences in preservation, and all were stuffed with cotton and/or plant fibre.

4.3 Results

Figure 2A shows the co-added XRF spectra for each specimen analysed in this study, which are plotted on a log scale of peak intensity in order to accentuate the peaks due to trace elements at low concentration. Specimens acquired by Broadbent (green), Grant working alone (orange), and Grant with Cairn (dark purple) were clearly differentiated by bulk elemental composition, specimens attributed to the same field collector had the same bulk elements present in similar concentrations This consistency was observed in skins collected during different expeditions with minor variation. This comparison required only the relative concentrations provided inherently by pXRF spectroscopy, therefore data were collected and presented as counts rather than absolute concentrations. These characteristics provided strong evidence that each of the collectors that are relevant to this study repeatedly used the same preservative mixture over time, *and* that the mixtures used by specific collectors differed from one another.

To further investigate, the Bayesian deconvoluted and Compton normalised XRF peak area data for all twelve pademelon specimens were compared using PCA. Elemental differences between the group collected by Broadbent, and those collected by Grant, and Grant with Cairn were revealed (Fig. 3). A total of 97.73% of the variance was described by principal component 1 (PC1) and PC2. Data from each location sampled on the specimens collected by Broadbent clustered along principal PC2 (6.29% of variance, Fig. 3, green). Neither expedition location, species, nor date had an impact in this analysis. Zinc (Zn), lead (Pb), and copper (Cu) distinguished specimens collected by Broadbent from other skins in the study (PC2 Loadings, Fig. 3B). Pademelon specimens collected by Grant with Cairn during both expeditions clustered along PC1(91.44% of variance, Fig. 3, purple) and were separated from the other specimens due to the presence of high relative concentrations of arsenic (As), as shown in PC1 Loadings Fig. 3B. Pademelon specimens collected by Grant alone (Fig. 3, yellow) during the expedition to Bellinger River NSW, clustered at the junction of the other groups due to a lower relative concentration of elements (ie the average of the elements). However, this observation is confounded because the pademelon species collected by Grant during the 1892 Bellinger River expedition (*T. thetis*) was different to those collected during the other expeditions examined in this study.

4.3.1 Broadbent

Within the group of specimens collected by Broadbent, Hg and Cu are correlated (Pearson's R^2 correlation is 0.72), which provided strong evidence that they were applied to the skins together (Fig. 4A). Both elements correlated to a lesser degree with Zn (Hg and Zn, $R^2=0.5$, Zn and Cu, $R^2=0.45$). While Pb was a distinguishing element for specimens collected by Broadbent when compared to spectra from other specimens, it was not correlated with Hg, Cu or Zn in the Broadbent specimens.

A further PCA of specimens collected by Broadbent only, illustrated that elemental variation in the skins formed three distinct groups observed via a 3D correlation loadings plot (Fig. 5A). The major variance described by PC1was dominated by Zn (75.05%), PC2 by As (12.72%), and PC3 by Pb (7.29%) as shown in Fig. 5B, C and D. Zn is an endogenous element in animals however, the relationship of Zn with Hg (shown above) demonstrated that the majority of Zn detected

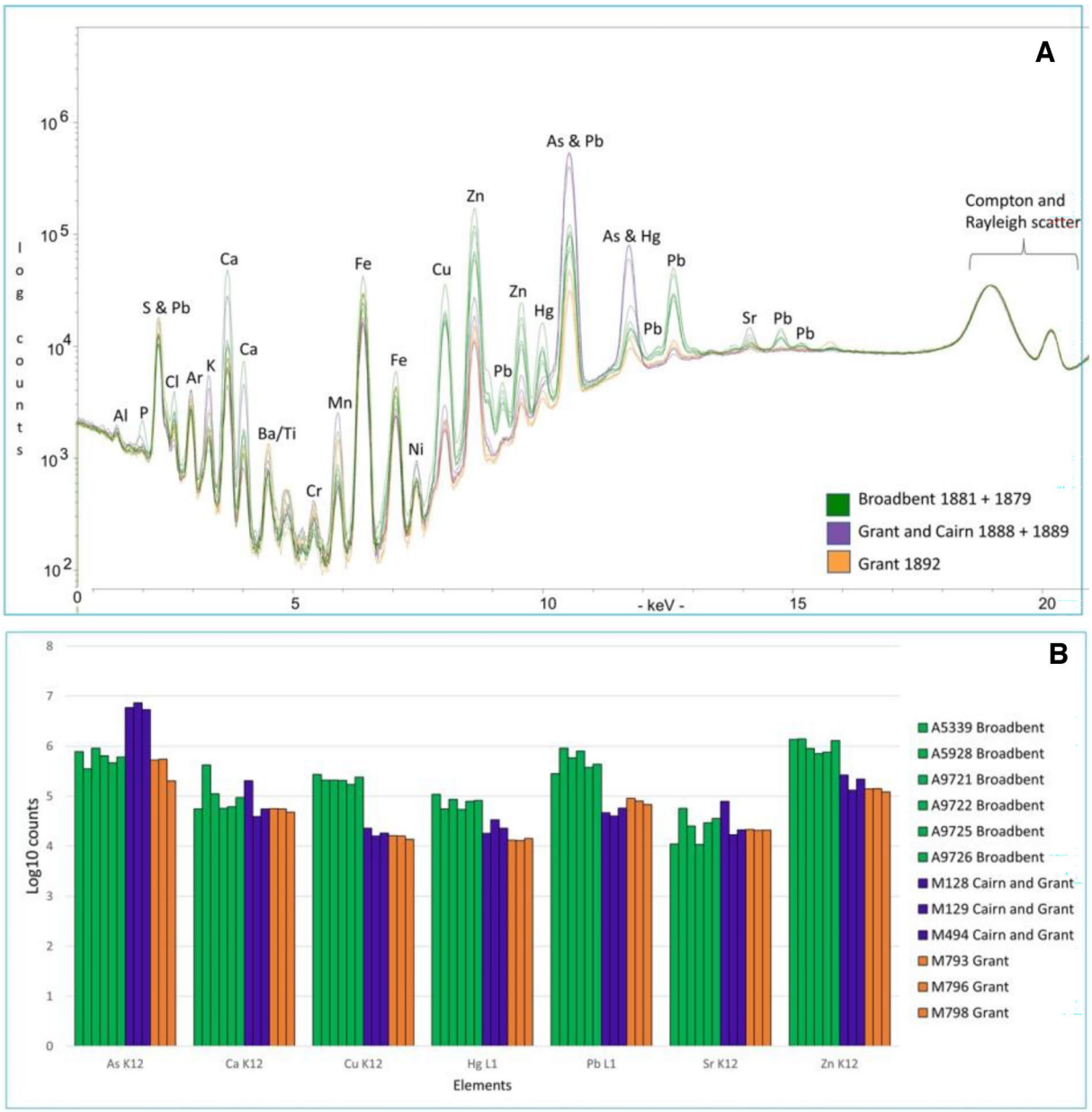

Fig. 4.2 **A** Comparison of co-added XRF spectra (log scaled) collected from twelve pademelons (*Thylogale*) showed that skins collected by each of: Broadbent, Grant, and Grant with Cairn formed distinct elemental patterns with copper, zinc, mercury, arsenic and lead immediately recognised as significant bulk elements. **B** Bar graph of significant bulk elements, derived from Compton normalised accumulated XRF spectra, showing that each field collector repeatedly used similar mixture on numerous specimens.

was exogenous and artificially applied with Hg. These three distinct sources of variation (in PC1, PC2 and PC3) were interpreted as residues from three different applications of chemicals. These applications may have all occurred at the time of field collection, or be a combination of field collection preservatives and pesticide treatments since the acquisition of the animal from the wild.

4.3.2 **Grant working with Cairn and alone**

The initial PCA that compared XRF data showed that pademelons acquired by Grant working with Cairn in both 1888 and 1889 expeditions had a higher relative concentration of As than those collected by Broadbent, and those collected by Grant alone (Fig. 3A). Additionally, a correlation between the As Kα peak (at 10.54 keV) and a Lα peak for thallium (Tl) (at 10.27 keV) was

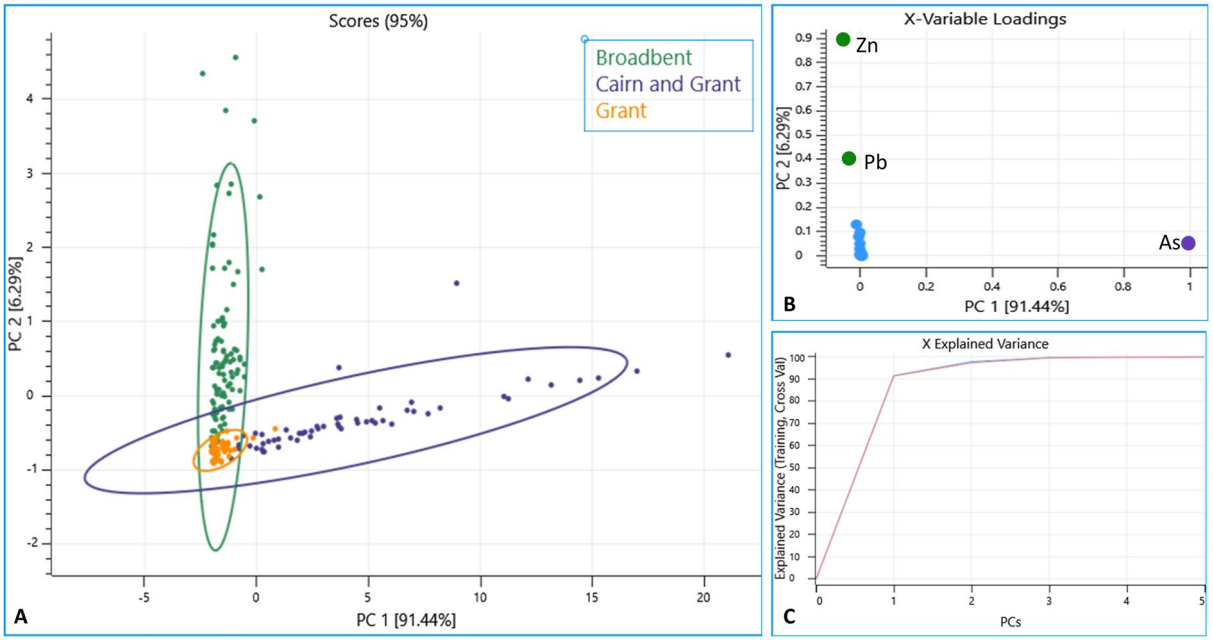

Fig. 4.3 Principal component analysis overview **A** PC1 versus PC2 scores plot shows clear differentiation between specimens collected by Grant, Grant with Cairn, from those collected by Broadbent. **B** The variable loadings plot shows the elements Zn and Pb describe Broadbent's specimens long PC2, while a high relative concentration of As describes specimens collected by Grant with Cairn along PC1. **C** The X-Explained Variance plot shows that two PCs explain over 97% of the XRF data for both calibration and cross-validation sets

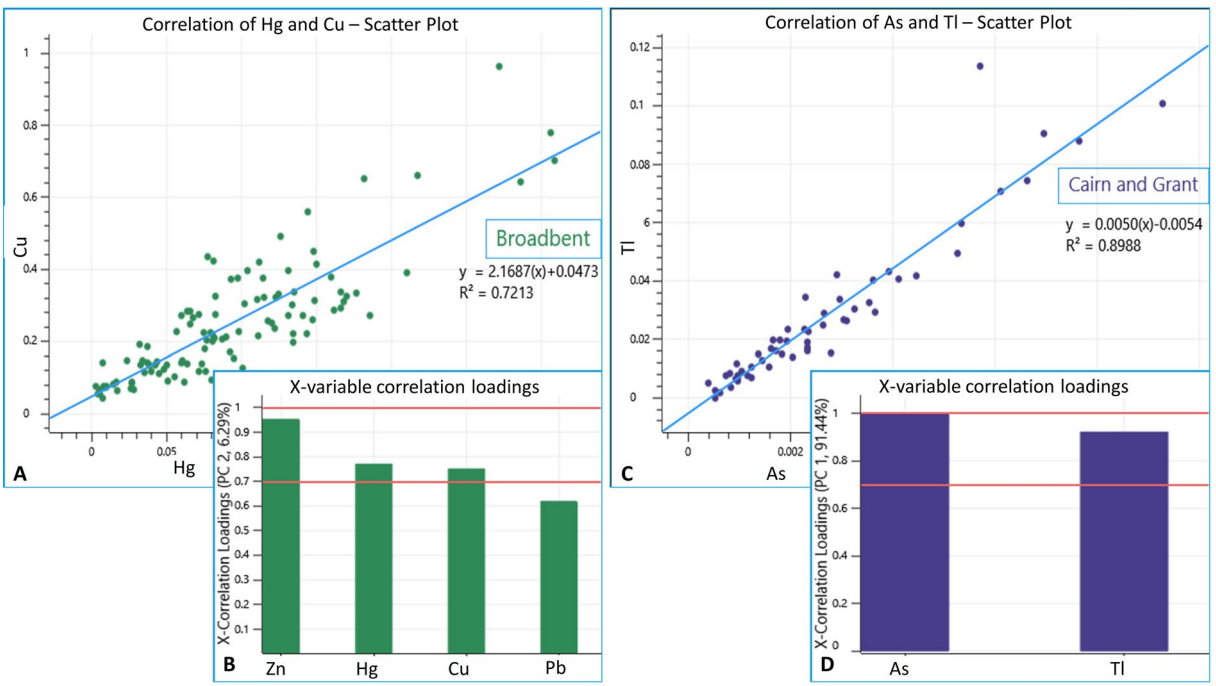

Fig. 4.4 Correlation loadings plot for PC2 showed that Zn, Hg, and Cu are correlated in specimens acquired by Broadbent (**B**), this is supported by Pearson's correlations (**A**). Correlation loadings plot for PC1 showed a correlation between As and Tl in specimens acquired by Grant with Cairn (**D**) (supported by Pearson's correlations **C**)

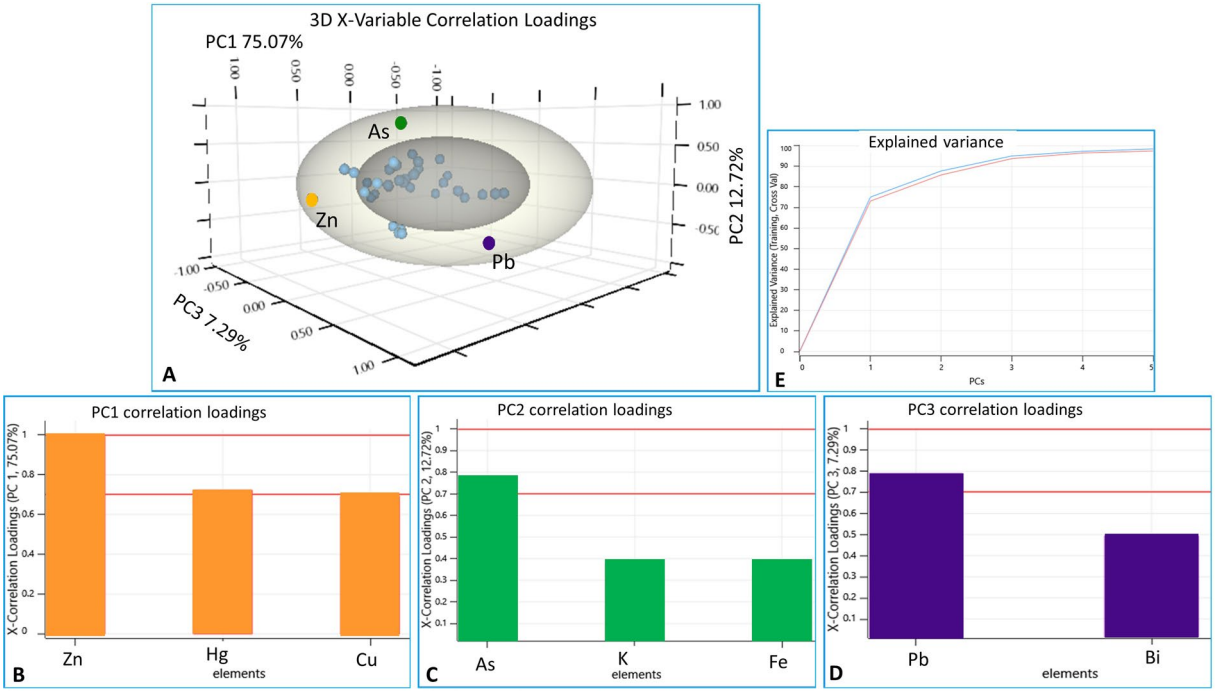

Fig. 4.5 PCA 3D correlation loadings plot showed three distinct groups of variation: **A** described by PC1, PC2, and PC3 (**B**-**D**) in the Broadbent specimens. (**E**) shows explained variance of the PCA of only the Broadbent specimens

identified in the Bayesian deconvoluted data for specimens collected by Grant working with Cairn ($R^2 = 0.89$) (Fig. 4C, D). Re-examination of the raw XRF data showed that Tl Lβ peaks did not correspond with the Tl Lα peaks in these samples indicating that Tl was not, in fact, present. Rather, a spectral overlap had been created around 10.3 keV by the very high As concentration in these specimens. Overlaps of elemental peaks occur in XRF spectroscopy in the presence of high concentrations of particular elements, causing misinterpretation during software run deconvolutions of the XRF data. This highlights that full reliance on software generated results, without human intervention to cross-check unexpected results, can lead to significant misinterpretations of complex data sets in chemical analysis [43, 92]. A cross-check to determine whether all of the required Tl peaks were present within the original data set, showed that the presence of Tl in the software generated assignment of elemental peaks as an artefact and hence, was removed in the interpretation of elemental differences.

A further PCA was therefore conducted to investigate the differences between pademelons acquired during Grant's expedition to the Bellinger River in 1892 and those from the two expeditions he undertook with Cairn. Variation between these two groups was explained by a single principal component (Fig. 6C). PC1 (99.82%) exclusively described variations in specimens collected by Grant with Cairn and was strongly dominated by As (Fig. 6A, B). Pademelons collected by Grant working alone in 1892 (Fig. 6A, B) were described by PC2 (0.08% of the variation). While Grant did use As in the preservation of *T. thetis* in 1892, the significantly higher concentration of As (95% confidence) in the *T. stigmatica* specimens collected by Grant and Cairn formed the greatest difference between the two groups. It was noted that this result was confounded with species variation therefore, a final analysis was undertaken to determine whether species independent information could be obtained.

When As was removed from this final analysis, more information was gained from the comparison of these two groups. Five of the six specimens analysed clustered mainly along PC1 (41.85% of variance) showing that aside from differences in As concentration, the elemental characteristics of the preserved specimens were similar (Fig. 7A, D, E). However, this was not the case for M.128, (collected by Grant and Cairn) in which relatively high concentrations of Ca and Sr were detected. This was described by PC2 with 25.3% of variance (Fig. 7A, B, C).

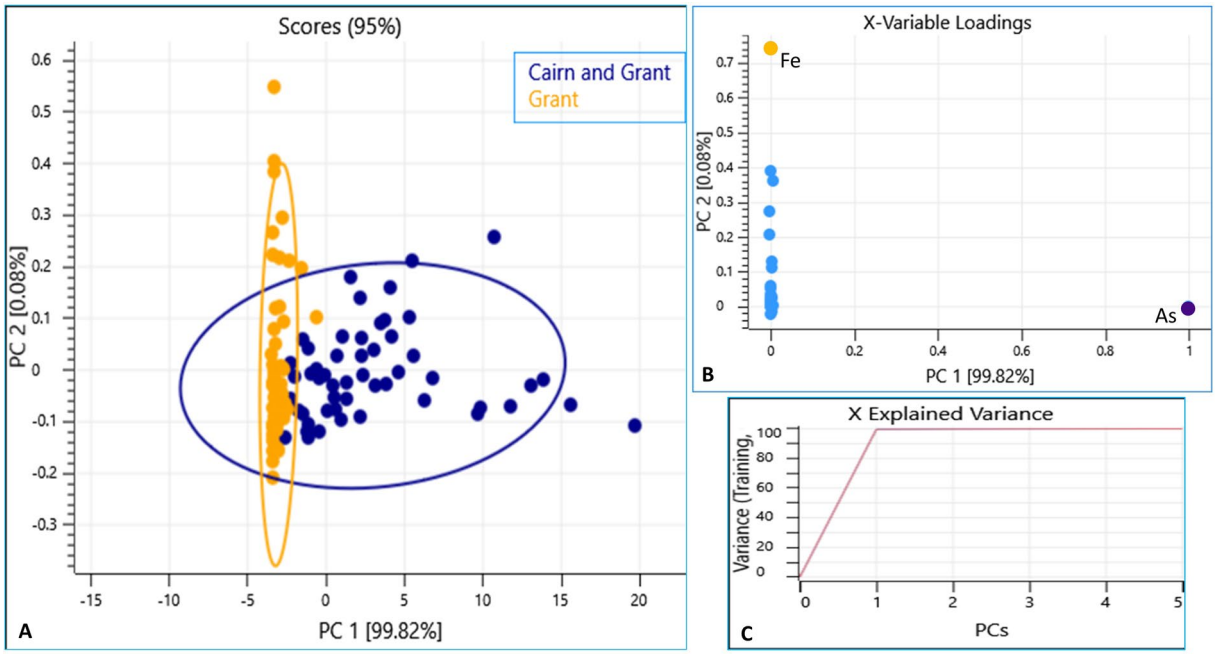

Fig. 4.6 PC1 versus PC 2 scores plot (**A**) showed specimens collected by Grant compared with those acquired by Grant when working with Cairn. **B** Specimens collected by Grant and Cairn were described predominantly by As (PC1) while specimens acquired by Grant working alone were mainly described by Fe (PC2). The X-Explained Variance plot (**C**) showed that a single principal component, PC1, explained the majority of differences
in elemental content between the groups

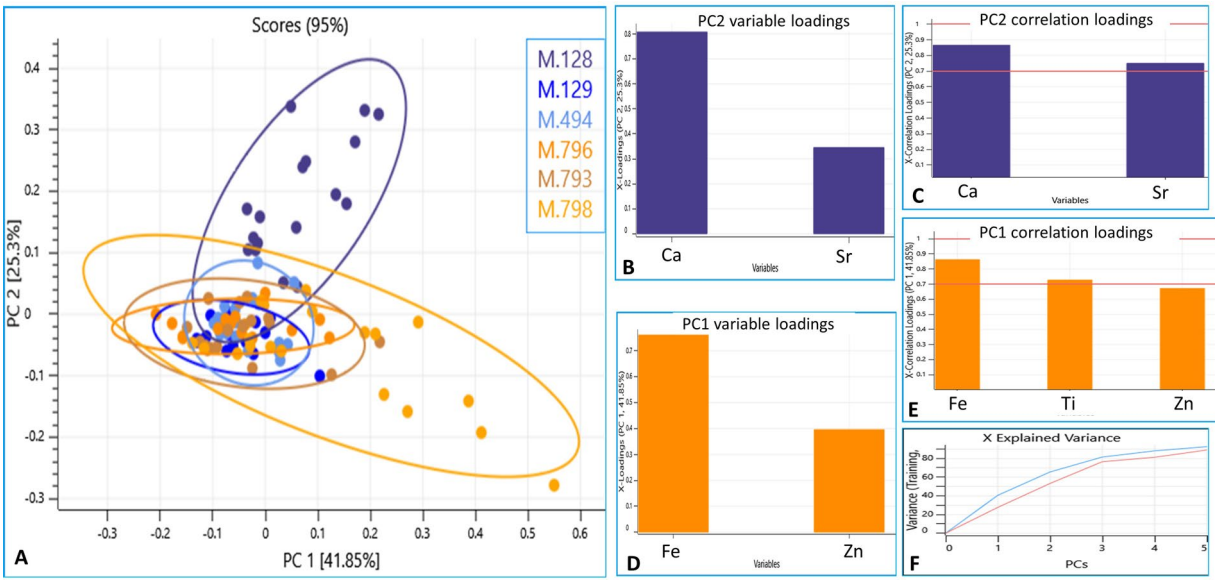

Fig. 4.7 When As was removed from the PCA, little difference was found between specimens collected by Grant with Cairn and those collected by Grant working alone in the PC1 versus PC2 scores plot (**A**). PC1 was dominated by Fe (**D**, **E**) and describes five of the six specimens. PC2 described only M.128 with Ca and Sr being important elements (**B**, **C**). Explained variance plot (**F**)

4.4 Discussion

Analysis of the XRF spectroscopic peak area data collected from the specimens investigated in this study revealed that each of the nineteenth century naturalists repeatedly used the same preservative chemicals, in similar proportions, over multiple expeditions, and over time. These patterns of practice were sufficiently different from each other, that groups of specimens acquired from the

field by each naturalist were differentiated (Fig. 3). In all specimens studied, neither the application of pesticides at unknown intervals in the history of the specimen, nor the contribution of elements from endogenous sources, such as Ca, Cu, Fe and Zn, confound the capacity to differentiate between collectors.

The data acquired from specimens collected by Broadbent showed that he used a similar mixture of chemicals as skin preservatives in Tasmania's cool climate (1879) and Queensland's hot tropical climate (1881) though the expeditions were in distinctly different climatic regions and with different access to local chemical supplies (Fig. 6). PCA of correlations within the XRF data provided strong evidence that the Broadbent specimens were chemically treated three times, once with lead, once with arsenic, and once with zinc in combination with mercury and copper (Fig. 5). These correlations suggest multiple chemical application "events" which may originate from the field-preservation process, or from preventive pesticide treatments undertaken in museums. However, the data, alone, was unable to describe a timeframe for these application events. With the information from this analysis, the archival documents were searched again and two new observations were made. Correspondence between Broadbent and his employer, Edward Ramsay, showed that both men went to some effort to ensure that Broadbent's preferred chemicals and materials were sent to him including "corrosive" [sublimate] (mercuric chloride) [93, 94]. Correlations (Fig. 5B) also provided evidence that copper was combined with mercuric chloride at application which indicated that these elements were ingredients in a preservative or pesticide applied to Broadbent's specimens. This was the first indication that our protocol may contribute specific information of Broadbent's field preservation practice. Additionally, the search of archival material related to Broadbent revealed a receipt for the purchase of chemicals [94] "arsenic alba", "salt tartar" (potassium carbonate), "camphor", and "rock lime", a list of ingredients matching to Ramsay's published arsenical soap recipe [95] but with quite different proportions. It was proposed that the chemical application event shown in Fig. 5C, in which arsenic and potassium are identified, may be related to the application of this arsenical soap. However, the configuration of the pXRF in this case limited detection of potassium and therefore the full relationship between arsenic and potassium may be masked – this will be investigated in a follow up study.

PCA showed that specimens collected by Grant with Cairn had a higher relative concentration of arsenic than the specimens collected by Grant working alone (Fig. 6). This may be due to the use of a different preservative mixture in the field. However, because the two *Thylogale* species in this comparison are not the same (*T. thetis* and *T. stigmatica*), there is a possibility each species may have been exposed to different pesticides in the museum. Further PCA was undertaken to resolve this potential confound. The removal of arsenic from the subsequent PCA showed a strong similarity between the elemental characteristics of the pademelons collected by Grant and those collected by Cairn and Grant together regardless of the different pademelon species represented. Thus, other than arsenic, the preservatives applied by Grant were very similar whether working with Cairn or alone.

At the same time, the pademelon juvenile specimen M.128, while visually similar to the other dry study skins attributed to Cairn and Grant's 1888 Bellenden Ker expedition, was revealed to be unique. Elevated levels of Ca and Sr detected in M.128 were attributed to elemental leaching from bone and tissue, and subsequent salt redeposition onto soft tissues, which was consistent with the use of formalin in preservation [96–98]. This observation, coupled with a general report of the Bellenden Ker expedition that showed only one juvenile mammal was collected [88], led to the conclusion that M.128 was initially wet preserved in 'spirits'. Although M.128 had a different history or preservative application, it exhibited the same high arsenic that was characteristic of other specimens collected by Grant and Cairn. This indicated that high concentration arsenic was applied to M.128 after it was removed from spirits, prepared as a dry specimen, and stored with the other *T. stigmatica* collected by Cairn and Grant. It was concluded the variation observed between specimens collected by Grant working alone and those collected by Cairn and Grant (which was characterised by very high As) was the result of pesticides applied at the museum and not a different preservative applied in the field. Thus, Grant's preservation practice did not change significantly when working with Cairn. This shows that the developed methodology when applied in this case study, can discriminate between specimens collected by different collectors *and* can clarify field-collection preservation practices over an individual collector's career. Equally important is the ability to determine the nature of variations in the sequence of preservation as a subset of the general pattern of preservation practice.

4.4.1 **Study limitations**

Three limitations should be considered when interpreting this study in the context of zoological museum collections. Firstly, a small number of specimens and field collectors were used which inherently limits the potential to apply the observations made here to other field collectors or global collection practices. Secondly, the instrument settings used in the collection of XRF data were set to optimise collection of fluorescence from elements with higher weights than manganese, therefore, reducing the

sensitivity to detection of light weight elements. Finally, the correlation analysis used for the Broadbent specimens in this study cannot provide the order of sequence for different preservative applications.

4.5 Conclusions

As specimen collecting decreases through climate change, and social, moral, and legal constraints on field collecting from the wild, museum zoological specimens are recognised as increasingly important, non-renewable resources [59, 99]. Hand in hand with this increasing importance, is a need to return museum specimens with limited use due to lack of contextual data, to the museum's collection of research ready material. To achieve this without physically sampling, non-destructive methods to establish provenance and object histories must be found. Based on the results presented in this proof-of-concept study, a protocol for applying pXRF and PCA has been designed and verified, which has several exciting potential applications for museum zoological collections.

By applying pXRF spectroscopy to twelve *Thylogale* study skins from the Australian Museum, and analysing all elements simultaneously via PCA, it has been demonstrated that the nineteenth century field collectors in this study, followed a consistent pattern of practice in specimen preparation, *and* that the specimens acquired by different field collectors were discriminated from one another. The capacity to recognise specimens as part of a group, and identify those that are unique, demonstrated the potential for this protocol to be applied to reunite unlabelled or mislabelled specimens with their legacy data, and to authenticate the provenance of culturally significant specimens, and other skin-based objects.

Using correlations within the data, this study has shown that separate applications of preservatives and pesticides, or contaminates, were distinguished as unique 'events' in the "afterlife" [100] of a specimen. Knowledge acquired through the application of this protocol, was used to identify specific animals w ithin g eneralised reports, and to recognise the significant documents within the archives. Furthermore, the capacity of the protocol to identify an individual skin that was once "wet" preserved in formalin from within a group of similar dry study skins was demonstrated. Thus, illustrating the value of this type of study to bridge gaps in the archival records of museum specimens, and a potential for application in determining the recipes applied to zoological specimens both in the field and in the museum.

This major advance in the application of pXRF spectroscopy in combination with PCA to differentiate preservation methods and materials used in the fieldwork of nineteenth century Australian naturalists opens new doors for augmenting geographical and temporal specimen data. This, in turn, broadens opportunities for investigating potentially important natural history specimens (e.g., extinct or locally extinct species) as well as enhancing the use of museum specimens in research including environmental, medical, and historical studies. The future application of this protocol may shine light on the often undocumented, methods and materials used in the preparation of zoological skins in the nineteenth century.

Abbreviations

As	Arsenic
Ca	Calcium
Cu	Copper
Fe	Iron
Hg	Mercury
PCA	Principal component analysis
PC	Principal component
Pb	Lead
pXRF	Portable X-ray fluorescence
Sr	Strontium
Ti	Titanium
Tl	Thallium
XRF	X-ray fluorescence
Zn	Zinc

Acknowledgements

The authors would like to acknowledge the kind and generous contributions of Sarah Kelloway, and Sandy Ingleby, Harry Parnaby, and Vanessa Finney of the Australian Museum. This research was facilitated by access to Sydney Analytical, a core research facility at the University of Sydney.

Author contributions

PXRF in-situ analysis was undertaken by CC. PCA of XRF peak area data was undertaken by CC and BS and interpreted by CC. JP and EC contributed to the conception of this work. JP facilitated access to museum specimens at the Australian Museum. EC, PL and JP provided academic supervision. CC wrote the first draft of the manuscript, all authors read, provided input to subsequent drafts and approved the final manuscript.

Funding

This research has been funded by the Australian Research Council, Linkage Grant LP1601761.

Availability of data and materials

The data set created and analysed during this study is available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare that they have no competing interests. Brad Swabrick owns the software used for the PCA, Vektor Direktor v1.1 and thus has a commercial interest in this study.

Received: 16 March 2023 Accepted: 13 July 2023
Published online: 25 July 2023

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Publisher's Note

Afterword

This chapter demonstrated, for the first time, that XRF data collected with a portable instrument, and analysed using PCA, could identify connections between specimens captured and preserved by the same field collector. While it provided strong evidence that there was a relationship between residues and field-collector, the limited number of specimens used in the analysis reduced the ability to make broader generalisations. The results reflected only the relationships between the specimens and field collectors in the study. There remained a possibility that the results were the product of an unknown event or other coincidence. Therefore, a further study using a broader selection of specimens and field collectors was required. The following chapters, Chapters 5 and 6, apply the XRF/PCA protocol first tested here in Chapter 4, to confirm the validity of the hypothesis made in this chapter.

Chapter 5

Spotting Spirit Specimens

5.1 Introduction

Chapter 4 described a method of discriminating between museum study skins collected by Kendall Broadbent (1837-1911) and those collected by Robert Grant (c.1854-1923) when working alone or with his colleague E.J. Cairn (unknown-1939) (1). This discrimination was based on protocols that incorporated the use of portable X-ray fluorescence (XRF) spectroscopy in combination with principal component analysis (PCA). It was concluded that this analytical protocol enabled attribution of study skins to historic field collectors or collecting groups. However, the inclusion of only three collectors limited the study from making broader observations about field collecting practices in nineteenth century Australia.

To gain a broader understanding of the relationship between preservative residues and field collection practices, a second study was designed and reported in this chapter. In this study, the protocol described in Chapter 4 (1) was applied to a larger group of museum specimens representing more collectors to determine if specimens could still be distinguished by their field collector. XRF data collected from fifteen *Thylogale* (*Marsupialia: Macropodidae)* skins from the Australian Museum were compared using PCA. These specimens were attributed to the collectors Kendall Broadbent, Robert Grant working with E.J. Cairn, Robert Grant collecting alone, Henry Sneddon Grant (c.1892-1944), John Archibald Boyd (1846-1926), and John A. Thorpe (unknown-1907). All the *Thylogale* study skins observed were visibly similar – all in good condition, kept in the same dry storage location, with light internal stuffing, and limbs were arranged in similar positions. However, like many older specimens in museums, records for these *Thylogale* study skins did not include information related to their histories as objects in a museum collection. Consequently, there was no information about the preservation

applied in the field, nor any reworking or pesticide treatments performed once in the museum (2,3).

The broader perspective of this study revealed an unexpected duality in the elemental composition of the specimens, and the surprising importance of zinc (Zn) in the residues of preservation. To determine the significance of zinc concentration, specimens from the Chau Chak Wing Museum's Macleay Collection, University of Sydney (MAMU) and study skins of the species *Marsupialia: Peramelidae: Perameles myosuros* (also named the Western Barred Bandicoot) from the Australian Museum (AM), were included in the analysis. This investigation using portable XRF and PCA revealed an association between those specimens that had been 'wet' preserved in alcohol and high relative concentrations of Zn and Cu found using portable XRF spectroscopy.

5.2 Experimental

5.2.1 Portable XRF Spectroscopy

Analysis was performed using a Bruker Tracer 5i portable XRF spectrometer (Karlsruhe, Germany; heretofore referred to as Tracer) at 40 kV and 30 μA for 40 s with a Ti 25 Al 300 filter and using an 8 mm collimator, in air. The Tracer was mounted on its stand beneath a custom-built specimen stage (see Section 2.3.1 Sample density, thickness, and surface irregularity)**.** The instrument was controlled using a laptop and Bruker ARTAX software (v8.0.0.476).

The specimens were analysed without any prior preparations. XRF spectra were collected *in situ* at the AM and MAMU, from at least ten distinct sites on the skins of each specimen. A minimum of two spectra were collected from the belly, back, left and right sides, and the head. The tail was not measured as internal wire supports present in some specimens, may be detected

by the instrument and influence the data collected. The specimen stage was cleaned with 70% ethanol and water, and disposable paper towel after the analysis of each specimen.

5.2.2 Data analysis

Spectra with dead times above 10%, or with poorly defined peaks, were excluded as they were considered unsuitable for reliable elemental analysis. ARTAX software (v7.8.2.0 Bruker) was used to conduct Bayesian deconvolution on the remaining XRF spectra, with escape and pileup peak, and background correction enabled in the peak area evaluation. Results were exported to a .xlsx format. Compton peak area was calculated for each spectrum using *region of interest* in ARTAX. Using Microsoft© Excel, the XRF peak area data were normalised to the Compton peak area (18.5-19.5 keV) for each spectrum. No further pre-processing was undertaken. The normalised data set was imported into VEKTOR DIREKTOR (v1.1 KAX Group, Australia) where PCA was performed using random cross-validation, and mean centring for 1000 iterations over five components.

5.2.3 Materials

The Australian mammal species selected for this study were chosen from the collection at the AM, in the first instance, for their size and ease of handling to ensure they could be measured with minimal risk of damage. *Thylogale* spp. are no larger than 1 meter in length, while *P. myosuros* are no larger than 35cm in total length. The second reason for selecting these species was the number of examples that could be attributed to field collectors with a well-documented history of supply or trade with the AM. Only the *Thylogale* spp. represented the work of more than four well known collectors and were numerous enough that more than one specimen could be selected from each of those field collectors and groups.

The mammal collection at MAMU was developed at a similar time as the AM collection. Thus, the same *Thylogale* species are represented within both collections, however data atrophy has left many specimens in this collection with limited provenance (4).

Table 5.1: *Thyloga*le spp. specimens from the AM, analysed in this study.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number
K. Broadbent	1879	*T. billardierii* ♀	Tasmania	Study skin	A.5339
K. Broadbent	1879	*T. billardierii* ♂	Tasmania	Study skin	A.5340
K. Broadbent	1879	*T. billardierii* ♀	Tasmania	Study skin	A.5342
K. Broadbent	1880	*T. thetis* ♂	Darling Downs, QLD	Study skin	A.9725
R. Grant & E.J. Cairn	1888	*T. stigmatica* juvenile	Bellenden Ker, QLD	Study skin	M.128
R. Grant & E.J. Cairn	1888	*T. stigmatica* ♀	Bellenden Ker, QLD	Study skin	M.129
R. Grant & E.J. Cairn	1888	*T. stigmatica* ♂	Bellenden Ker, QLD	Study skin	M.133
R. Grant & E.J. Cairn**	1889	*T. stigmatica* ♂	Herberton district, QLD	Study skin	M.494
R. Grant & E.J. Cairn**	1889	*T. stigmatica* ♀	Herberton district, QLD	Mount	M.496
R. Grant	1892	*T. thetis* ♀	Bellinger River, NSW	Study skin	M.794
R. Grant	1892	*T. thetis* ♂	Bellinger River, NSW	Study skin	M.796
H.S. Grant	1913	*T. thetis* ♀	Barrington River, Bulliac NSW	Study skin	M.2320
H.S. Grant	1913	*T. thetis* ♂	Barrington River, Bulliac NSW	Study skin	M.2321
J.A. Thorpe (Received by donation)	1888*	*T. stigmatica* ♂	Richmond River, Ballina NSW	Study skin	M.203
J.A. Boyd (Received by donation)	1895*	*T. stigmatica* ♂	Herbert River, QLD	Study skin	M.957

*This date represents the specimen entering the AM collection, it may not be the year of collection from the field.

**Although registered in the AM records as collected by Grant and Cairn, the report from the collecting trip shows Grant did not accompany Cairn to the Herberton District (5).

Table 5.2: *Thylogale* spp. specimens from the MAMU collection, analysed in this study.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number
Unknown	Unknown	*T. stigmatica* ♂	Herbert River, QLD	Study skin	NHM.408
Goodman	1879*	*T. stigmatica* ♀	Richmond River, NSW	Study skin	NHM.429
Goodman	1879*	*T. stigmatica* ♀	Richmond River, NSW	Mount	NHM.430
Goodman	1879*	*T. stigmatica* ♂	Richmond River, NSW	Mount	NHM.431
Unknown	Unknown	*T. billardierii* ♀	Tasmania	Mount	NHM.437

*This date represents the specimen entering the MAMU collection, it may not be the year of collection from the field.

The field collection practices of Masters were not well represented in the *Thylogale* spp. specimens. Although Masters was a prolific field collector and contributed more than 645 mammal specimens to the AM collection, only the species *P. myosuros* were both small and had more than three study skin specimens remaining in the collection - Masters, himself, was surprised by the scale of trade in his specimens (6).

Table 5.3: *Perameles myosuros* specimens from AM and MAMU collections.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.435
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.436
G. Masters	1868	*P. myosuros* ♂	Salt River, WA	Study skin	P.437
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.440
Unknown	Unknown	*P. myosuros* ♀	King Georges Sound, WA	Mount	NHM.436

Fig:5.1A: "Halmaturus stigmaticus, *Gould*" (now named *Thylogale stigmatica*) (7 p.146).

Fig:5.1B: "Perameles myosurus, *Wagn*." (now named *Perameles myosuros*) (8 p.94).

5.3 Results and Discussion

5.3.1 Two clusters of specimens

The XRF data collected from the fifteen *Thylogale* skins were analysed using PCA. Analysis of this larger and more diverse data set revealed two distinct groups (Fig. 5.2). Scores plots in Fig. 5.2A and B, show how these groups clustered along the PC1 (Group A) and PC2 (Group B) axes, respectively. Over 99% of the data variance was described in two PCs (see explained variance plot Fig. 5.2C). PC1 accounted for 93.41% and described the key differences between the two groups. Most data in Group A had positive values in PC1 and were described by high relative concentration of arsenic (As), as shown in Fig 5.2D. The data in Group B had mostly negative values in PC1 and Zn, copper (Cu) and lead (Pb) were the main contributors to the loadings plot as shown in Fig. 5.2E. PC2 provided a more detailed description of Group B showing variation in Zn concentrations, as well as Cu and iron (Fe) in the loadings plot Fig. 5.2F, and accounted for 6.01% of variance.

To further investigate the two data groups observed in Fig 5.2, the median for each element was calculated from the 15-20 XRF measurements collected from each *Thylogale* specimen.

PCA of the median data set is shown in Fig. 5.3 in which individual specimens are represented by a single point in the scores plots Fig. 5.3A and B. Outlier points seen in Fig. 5.2A and B were removed using this median data and two clusters, Group A (red) and Group B (blue) were identified in Fig. 5.3B. Over 99% of variance is described by PCs 1 and 2 (explained variance plot Fig 5.3C). PC1 accounts for 93.15% of the data and describes Group A by As while Group B is described by Zn, Cu and Fe (Fig. 5.3A, D).

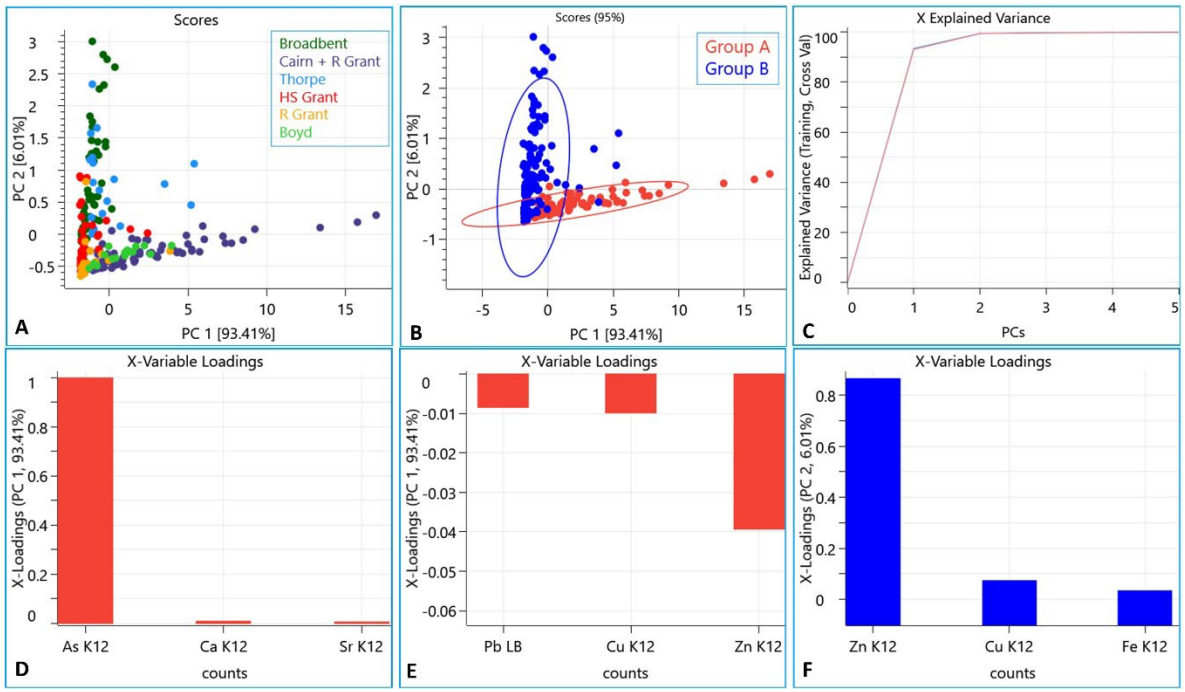

Fig. 5.2: Principal component analysis of *Thylogale* skins from the AM Two distinct groups were observed in the scores plot (**A**, **B**) with ellipses showing 95% confidence in (**B**). PCs 1 and 2 accounted for over 99% of the variance in the data set (explained variance plot **C**). The largest variance (93.41%) was described by PC1 with positive values describing As and negative values describing mostly Zn (loadings plot **D** and **E**). PC2 described the specimens mostly by Zn, Cu and Fe (loadings plot **F**).

The reason for the distinct grouping of these visibly similar specimens was not immediately clear. It was hypothesised that the two clusters were indicative of a broader duality in the

specimen preparation. Arsenic was used in dry and wet field preservative mixtures and as a pesticide applied to skins while in museum collections (3). Identification of this element as a major component in analysis was predicted. However, the significance of Zn in specimen preservation was not as easily interpreted.

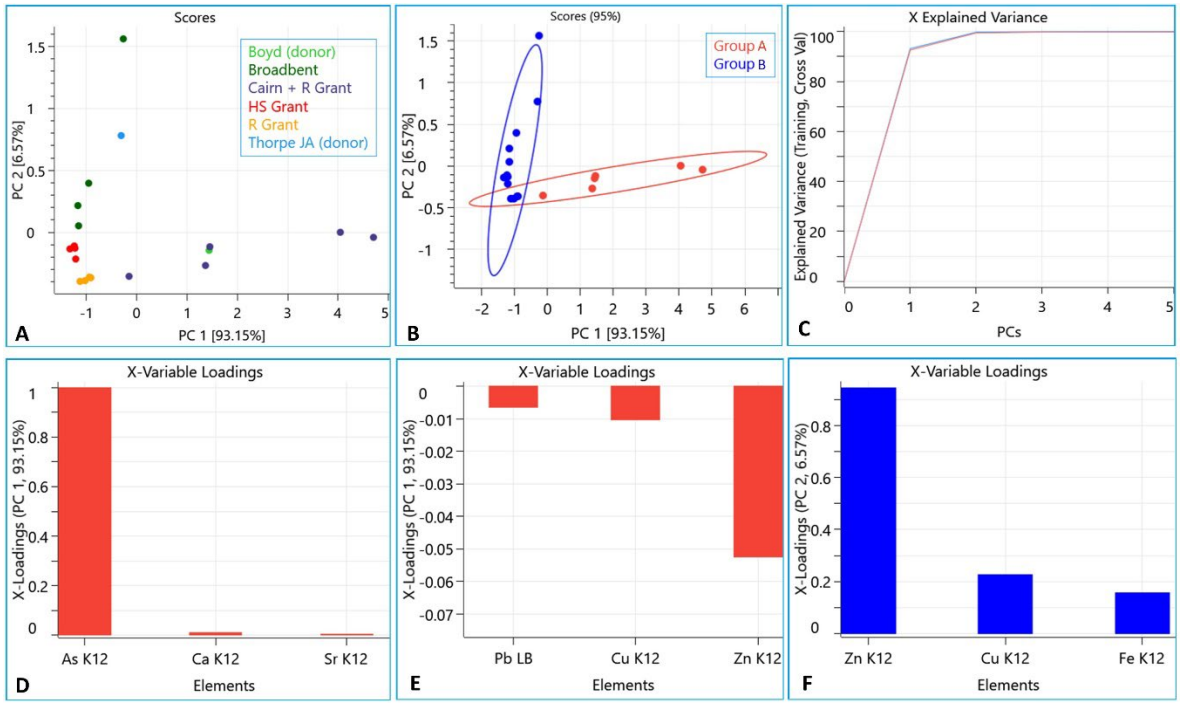

Fig. 5.3: PCA of median XRF data for Thylogale skins. Each of the specimens are represented by a single dot in (**A)** and (**B)**. The two clusters, Group A and Group B are identified in (**B)** using ellipses showing 95% confidence. Over 99% of variance is described by PCs 1 and 2 (explained variance plot **C**). PC1 accounts for 93.15% of the data and describes Group A by As, while Group B is described by Zn, Cu and Pb (**D, E**). PC2 further described Group B (**F**).

5.3.2 Investigating the significance of Zn

While Zn is an important trace element within skin and hair (9-12), such a significant variation in concentration between animals of the same species was unexpected. Fig. 5.4A, presents the scores plot for PCA of median data for each of the fifteen *Thylogale* skins (Fig 5.2B) colour

coded according to the specimen subspecies. The Group B specimen cluster, described mainly by Zn in Fig. 5.3B, included all three subspecies in the study with individuals from the diverse environments of Tasmania, inland NSW, and Northern Queensland. Therefore, subspecies (skin and hair thickness) and field collection location (thus endogenous environmental variations) did not account for the separate groups.

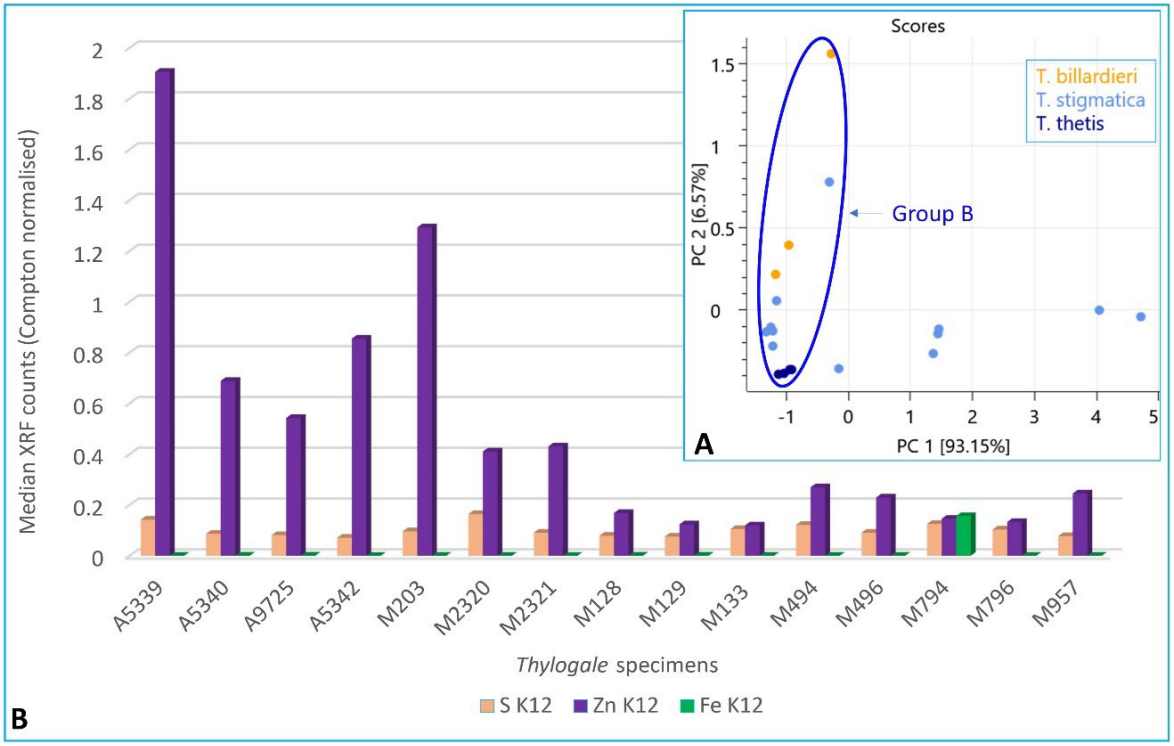

Fig. 5.4: (**A**) The PCA scores plot of median data first shown in Fig 5.3 coloured according to sub-species. Individuals from all three *Thylogale* species were included in Group B (clustered along PC2). (**B**) Bar graph of the median XRF counts for each skin shows the intensity count of Zn relative to S, and Fe.

To examine the factors affecting the intensity of Zn counts, Compton normalised XRF counts for Zn were compared with another element typically found in skin and hair, sulfur (S) (Fig. 5.4B). The counts for S were similar across all fifteen *Thylogale* skins. However, specimens identified in the PCA as members of Group B, A.5339, A.5340, A.5342, A.9725, M.203,

M.2320 and M.2321, had more than double the proportion of Zn compared to S. This demonstrated that the high concentration of Zn characteristic of the Group B specimens is identifiable using the counts data alone and suggests that relationship between Zn and S may be used to identify specimens that are similar to Group B without the use of PCA.

Counts for Fe were also scrutinised in Fig.5.4B to determine that the Zn levels were not affected by the presence of iron in a sample (13). The concentration of Fe in all samples (except M.794) was found to be low. Thus, for these specimens, the absorption of Zn fluorescence by Fe was not significant enough to affect the potential use of Zn to S ratio for identification of Group B specimens.

5.3.3 Historic use of Zn in specimen preservation

Zinc is not identified in the literature as an important or commonly used preservative. However, zinc chloride was recommended as a disinfectant and a deodoriser (14,15) and was used as an additive to wet preservative and tissue fixing recipes in a number of manuals of the mid to late19th century, including Browne's *Practical Taxidermy* (16) and Herschel's *Manual of Scientific Enquiry* (17) (18). Also known as 'Burnett's Disinfecting Fluid', 'chlorinate of zinc' and 'white vitriol' (19), zinc chloride was reported to have been used in the wet preservation of zoological specimens at the British Museum (20,21), Haslar Hospital Collection (22), and the Muséum d'Histoire Naturelle, Bordeaux (23). Gregerson proposed that zinc chloride was used as an inexpensive alternative to spirits of wine (24). The fixative, zinc sulfate, may also be a source of zinc in historic specimens (18,25,26). Another possible source is Zn leaching into preservative solutions from metal labels attached to specimens preserved in fluid (27), or from the vessels and tubs that contained solutions of pickles and preservatives used during preservation (28) (29).

The documentary evidence, therefore, suggested a connection between high concentrations of Zn and wet preservation methods, yet each specimen in this study was physically dry. A choice between two methods of preservation in the field is found in the manuals of the nineteenth century, creating "made" skins (30) or wet preserved specimens (16,17,31-33). A survey of the annual reports from the AM showed that the field collectors in this study sent both dry skins and wet 'spirit' specimens to the museum. For example, R. Grant and E. J. Cairn prepared 28 dry skins and 39 specimens 'in spirits' of numerous species for the AM during their expedition to northern QLD in 1888 (34).

5.3.4 Considering pesticides as a source of Zn

One final possible source for zinc could have been pesticides applied to the specimens while in a museum collection. Rodenticides and insecticides containing zinc phosphide and zinc hexafluorosilicate were developed during the twentieth century and were included in the commercially available products *Arrex*, *Ridall-z*, *Berlou*, and *Arko mothproof* (35). While no records of these pesticides has yet been found within the AM archives, soil studies from Coffs Harbour (in the same administrative and legal jurisdiction as the AM) provide evidence that such pesticides were in use in Australia from the 1930s (36). It was expected that, if these zinc-containing pesticides were present within the Group B specimens, high concentrations of Zn would correlate with high concentrations of the other elements within the pesticides *i.e.*, phosphorus (P) and silicon (Si). The data was interrogated to determine if any such correlation existed.

The scores plot originally presented in Fig. 5.3A, was colour coded according to the Compton normalised counts of Si in Fig. 5.5A, and P in Fig. 5.5B. XRF measurements from samples that had high Si or P levels did not correlate with high Zn levels in these plots. Measurements from the specimens with high concentrations of Zn (Group B, A.5339, A.5340, A.5342, A.9725,

M.203, M.2320 and M.2320) were analysed using the coefficient of determination. In Fig. 5.5D, very weak correlation was detected between Zn and P (R^2 = 0.1283), which discounted zinc phosphide as the source of Zn. In Fig. 5.5C, a weak correlation was found for Si (R^2 = 0.3253), however Si is a component of the instrument's detector, therefore, interpretation of this observation was made with caution. To further examine if the source of Zn was associated with twentieth century pesticides, the fifteen *Thylogale* specimens from the AM were compared to five *Thylogale* (study skins and mounts) and one *Perameles myosuros* mount from

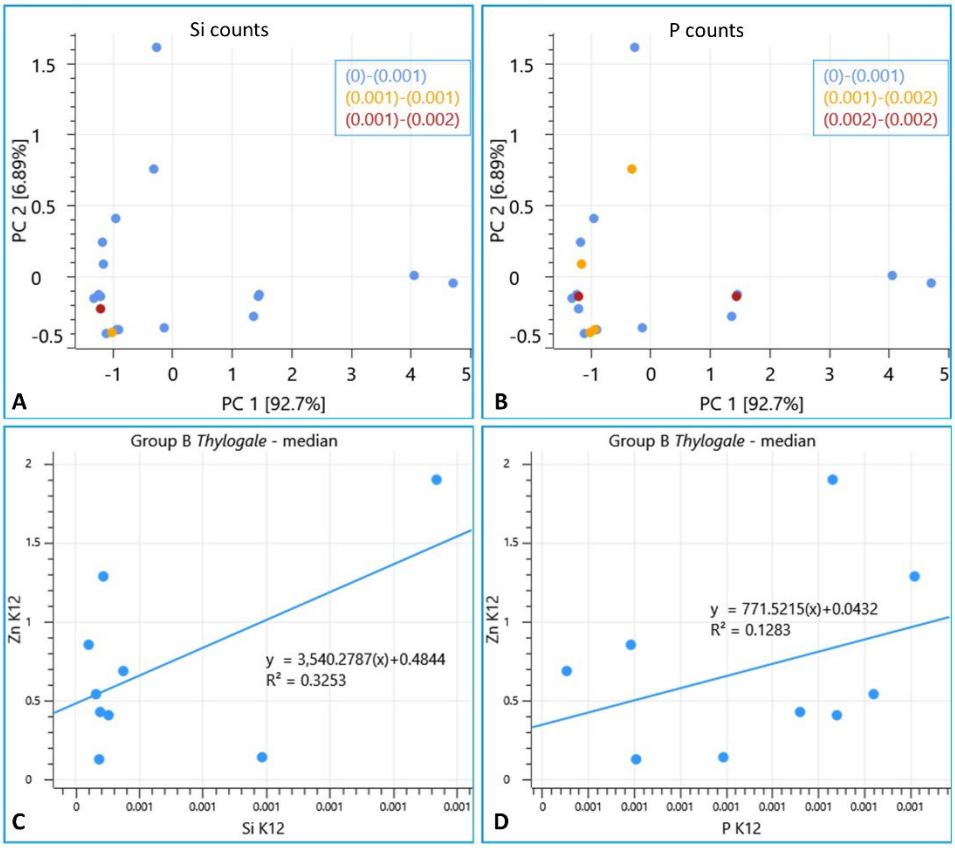

Fig. 5.5: The PCA scores plot first seen in Fig. 5.2A was recoloured according to Si counts (**A**) and P counts (**B**). Relationships between Si and Zn for specimens with high concentrations of Zn (A.5339, A.5340, A.5342, A.9725, M.203, M.2320 and M.2320) using Pearson's correlation (**C**). Relationship between P and Zn for A.5339, A.5340, A.5342, A.9725, M.203, M.2320 and M.2320 using Pearson's correlation (**D**).

the MAMU collection. These collections were acquired from similar Australian sources but were housed and managed separately (37). Thus, the two collections were unlikely to have been subjected to identical pesticide regimes during the twentieth century (38).

PCA revealed that the MAMU specimens also formed two clusters (scores plots Fig. 5.6A) and those clusters were more clearly seen when the median for each specimen was acquired (Fig. 5.6B). One cluster, circled in red, was dominated by As (loadings plot Fig. 5.6D). The other was dominated by Zn (described by PC1 loadings plot Fig. 5.6E, and further described by PC2 loadings plot Fig. 5.6G). Again only 2 PCs were required to explain the variance across the six specimens (explained variance plot Fig 5.6H). The PC1 correlation loadings plot, Fig 5.6F, showed that As correlated with Rb in the specimens that clustered above 0 along PC1. The two clusters, with similar elemental characteristics, found in the study of MAMU specimens suggested that the variations in pesticide applications and contaminates across the two distinct collections does not account for the two groupings.

It is interesting to note that Fig. 5.6C shows that mounts and study skins appear in both groups which speaks to the importance of initial preservation. Mounted specimens are subjected to two sets of circumstances that one may expect to produce elemental compositions that significantly differ from study skins. The first is the complex set of processes and components used to mount preserved skins and form taxidermy ‘display’ specimens. The second are the measures undertaken to protect mounted specimens from contamination from pests while on public display. Unlike the restricted storerooms for study skins, mounted specimens are subjected to pesticide treatment methods suited to the confined spaces of display cases. The differences in specimen form did not account for the distinct clusters in the MAMU specimens, indicating that neither taxidermy nor display pesticide applications are the source of high concentrations of` Zn in these specimens.

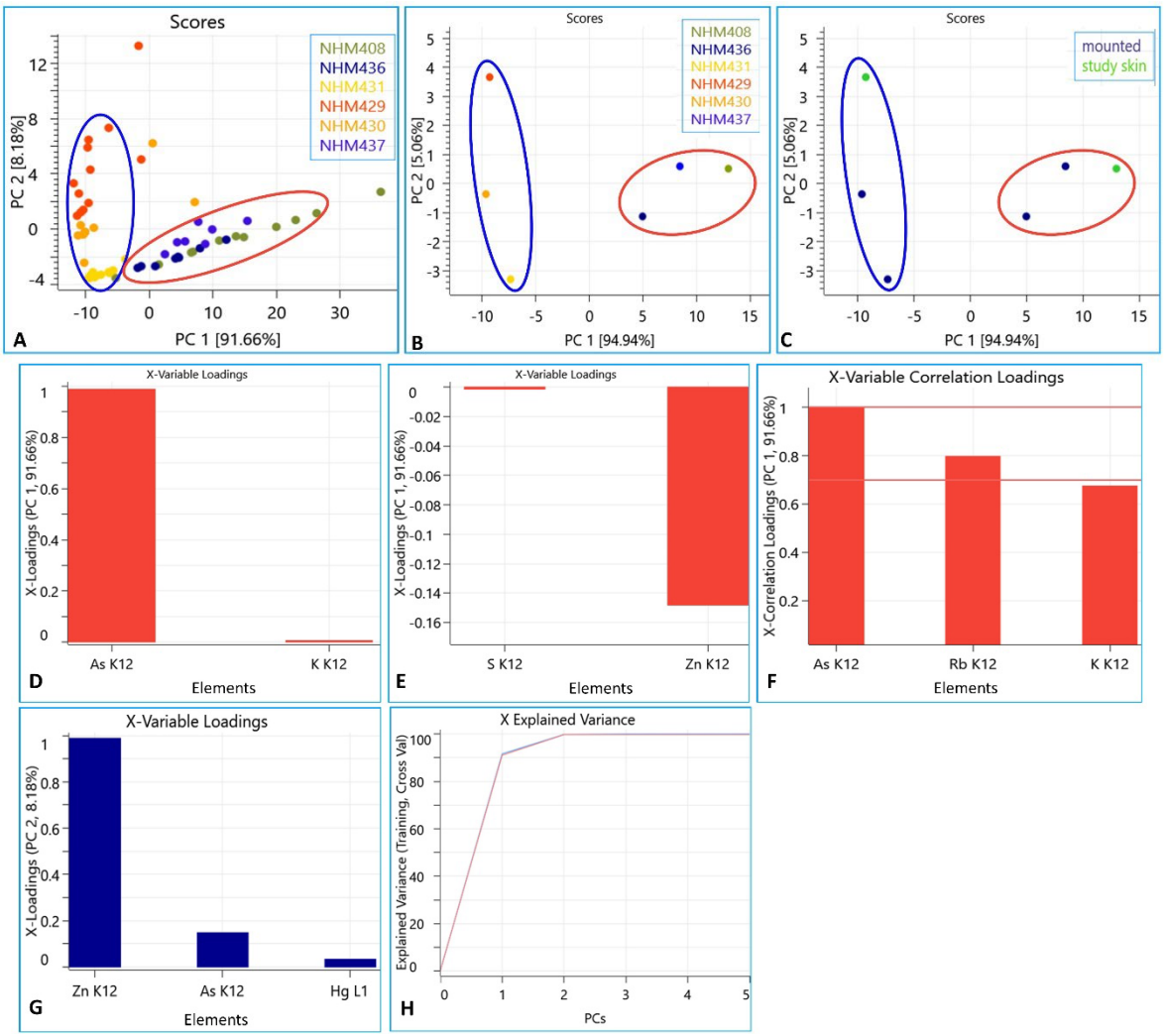

Fig. 5.6: PCA overview of XRF measurement from six specimens from the MAMU collection. Similar to AM specimens, they formed two groups, Group A identified by a blue ellipse and Group B identified by a red ellipse (scores plot **A**). These groups were more clearly separated when median data for each specimen was used (scores plot **B**). Mounts and study skins appear in both clusters (scores plot **C**). Both groups were described by PC1 positive values associated with Group A and described by As, while Group B had negative values, and was described by Zn (**D**, **E**). Arsenic was correlated with rubidium (Rb) in PC1 (correlation loadings plot **F**). PC2 further described Group B by Zn, As and Hg (**G**). Over 99% of variance was explained in two PCs (**H**).

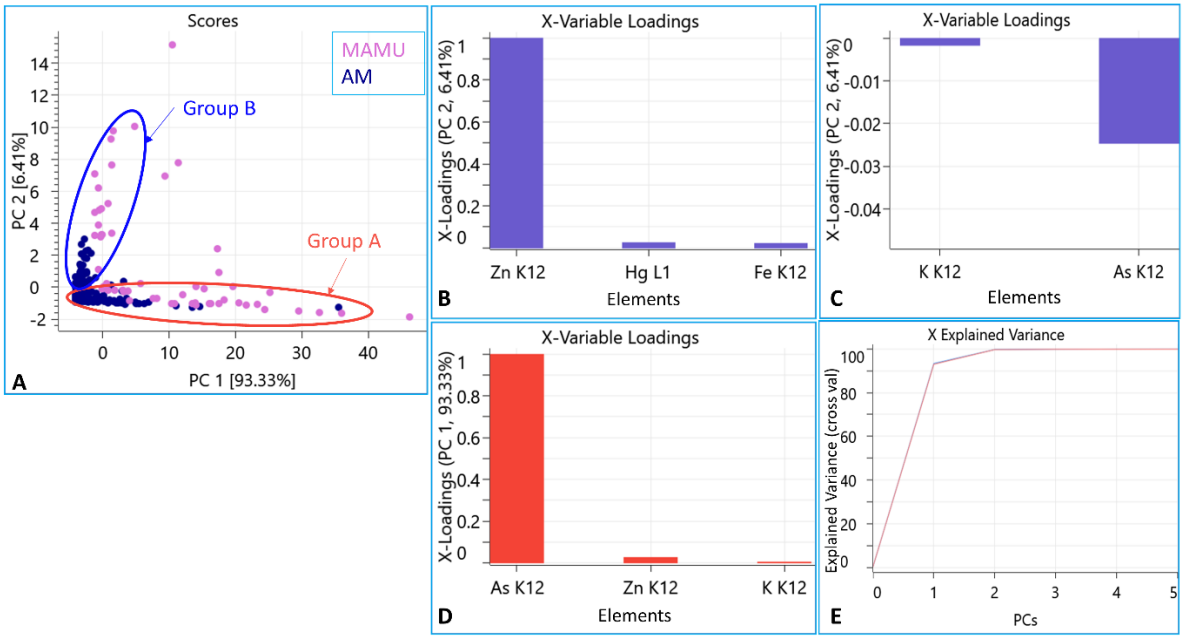

Fig. 5.7: PCA of *Thylogale* spp. specimens from the AM together with six specimens from the MAMU collection, scores plot (**A)** with ellipses added to show the two groups. PC2 describes the difference between the two groups (**B**, **C**). PC1 is dominated by As (**D**). 99% of variance was described by PCs 1 and 2 (**E**).

When specimens from both museums were analysed as one data set, two clusters remained identifiable as seen in the scores plot Fig. 5.7A. Again, one group was mostly described by As and the other mostly by Zn. PC2 described both Group A and B. Negative values for PC2 described Group A with arsenic as the main contributor to the loadings plot (Fig. 5.7C). Positive values for PC2 described Group B with Zn as the main contributor to component loadings (Fig. 5.7B). Interestingly, the spread of MAMU specimens plotted further along PC1 (positive values in PC1) than the AM specimens, indicating a higher concentration of As within that collection. The presence of a Zn dominated clusters in the data collected from both museums, coupled with the absence of reliable correlation between Zn and other elements of known zinc-containing pesticides (P and Si), led to the inference that that the source of the high

relative concentrations of Zn in the Group B specimens was not attributable to pesticides applied in the maintenance of museum collections.

5.3.5 Broadening the study to resolve the origin of Zn

Clarity on the cause of high Zn concentration was found through broadening the study to include a group of four bandicoot study skins (*P. myosuros*), from the AM collection, with archival evidence of a history of wet preservation. These specimens (P.435, P.436, P.437, P.440) were originally part of a larger group of twenty-two *P. myosuros* collected during the expedition to Salt River in Western Australia (now called Pallinup River) by Masters in 1868 (39,40). Twenty specimens were described in the annual report of 1868 as preserved in spirits, and another two preserved as dry skins. While the *P. myosuros* were not catalogued upon entry into the museum collection in the 1868, by 1880, all four were described as specimens "in spirits" in the museums "P register" (41) (Fig. 5.8). In 1912, paper labels were attached to P.440 and P.435, inscribed with "5/10/12 no data". This is evidence that both these former "spirits" specimens were no longer in spirit by 1912. In 1984, all four specimens were described as study skins with separated skulls. The archives show these specimens were initially wet preserved and were changed to dry study skins.

XRF data was collected from all four *P. myosuros* in the same manner as the previous specimens. Each of the measurements collected form these specimens was plotted to illustrate the compositional variation across the skin of each animal. The PCA scores plot (Fig. 5.9A) showed the specimens clustered into one group in which each specimen was described by both PCs1 and 2, confirming the similar object history provided by the archival evidence. Over 97% of the variance was explained by PCs 1 and 2 (Fig 5.9F), and the corresponding loadings plots in Fig 5.9B, D, and E, illustrate that numerous measurements from each specimen are described by both As and Zn.

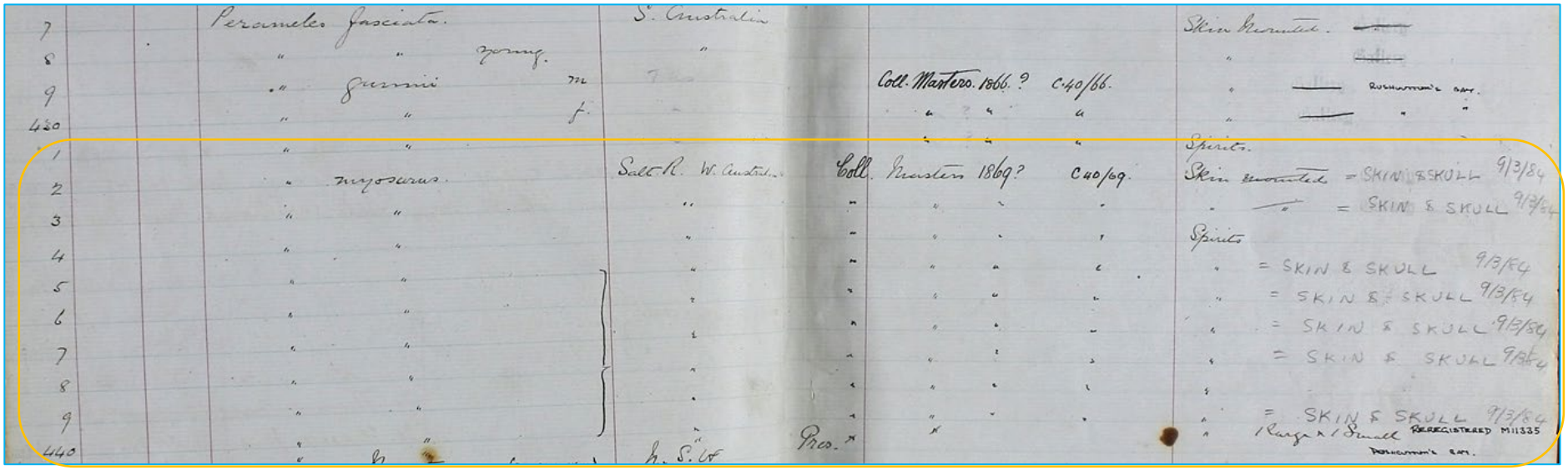

Fig. 5.8: Photograph of the AM's Palmer (P) register showing 1879 entries for the *P. myosuros* specimens P.435, P.436, P.437 and P.440. The original inscription, in fine black ink, show these specimens were in spirits. A pencil inscription, dated March 1984, describes them as dry skins with skull separate (41).

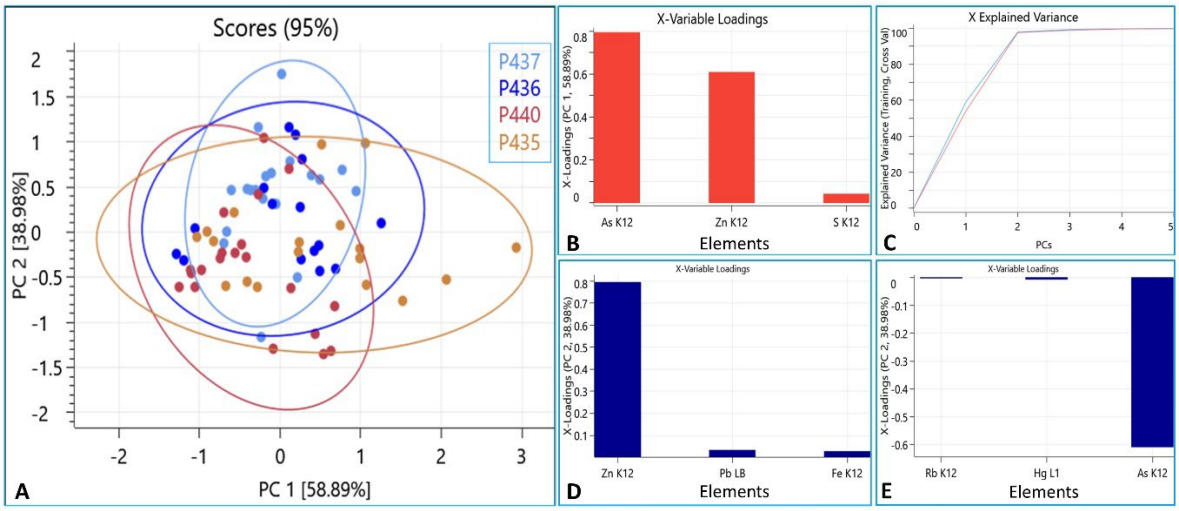

Fig. 5.9: PCA of XRF data collected from *P. myosuros* study skins with a known history of wet preservation. Scores plot (**A)** shows the specimens form a single cluster. Corresponding loadings plots detail the elemental similarity (**B**, **D**, **E**). Over 97% of the variance is explained by PCs 1 and 2 (**C**).

The XRF data collected from the four *P. myosuros* specimens were then projected onto the PCA model established using the AM *Thylogale* spp. skins and presented in Fig. 5.2. Unlike a typical PCA model, this projection described the *P. myosuros* data set using the principal components found to be important in the *Thylogale* data set. The purpose was to determine if the group of *P. myosuros* might help to interpret the two clusters seen in the data collected from the *Thylogale* specimens. As seen in the explained variance plot, Fig. 5.10B, more than 90% of the variance within the *P. myosuros* data was described in the same two PCs that described the *Thylogale* data. The *P. myosuros* specimens were predominantly described by PC2 loadings plot in Fig. 5.10C (53.83% of variance within the data) and to a lesser degree by PC1 loadings plot in Fig. 5.10D, E (39.48% of variance) showing that the projected samples have more in common with PC2 than PC1. The *P. myosuros* specimens were described mostly by Zn and Cu. All four *P. myosuros* specimens coincided with the Group B *Thylogale* spp. identified in Fig. 5.2B.

It was concluded that the high relative concentration of Zn described by PC2 throughout these analyses was due to a history of wet preservation. This finding is supported by Gibbs, *et al.*, who found elevated concentrations of Zn in fish specimens preserved in alcohol (27). Contaminates in commercially produced alcohol mixture were identified as the source of Zn. Interestingly, Zn and Hg concentrations were reported to increase with the length of time the specimen remained in alcohol. In a later study, Levengood *et al.* also showed a relationship between storage in alcohol and increases in Hg concentration (42). The well-established practice of topping up or replacing alcohol-based preservative fluid in response to natural evaporation and to maintain appropriate alcohol concentration and pH (43,44), may have repeatedly introduced trace metal contaminates to the solution making metals available to be taken up by tissues. Thus, the very high relative concentrations of Zn seen in museum wet preserved tissues may be attributed to storage in alcohol with, or without, the addition of Zn based additives, such as zinc chloride.

Unlike alcohol, long term storage in formalin has been shown to leach Zn and Cu, as well as iron (Fe), rubidium (Rb), calcium (Ca), chlorine (Cl), and potassium (K) in a range of different mammalian tissues (45-49). Conversely, short term fixation in formalin has been shown to increase Zn, Cu, and Fe due to contaminates in buffered formalin solutions (50,51). Both Gellein *et al.*, and Pushie *et al.*, attribute the effects of formalin to the nature of covalent bonding within biomolecules suggesting that elements covalently bound to proteins are less affected by preservatives and fixatives (48,51). This literature suggested that specimens exposed to long term preservation in formaldehyde/formalin may be distinguished from those preserved in ethanol by comparatively low concentrations of Zn, Cu and Fe, and led to the recognition that Group A may include specimens with such object histories.

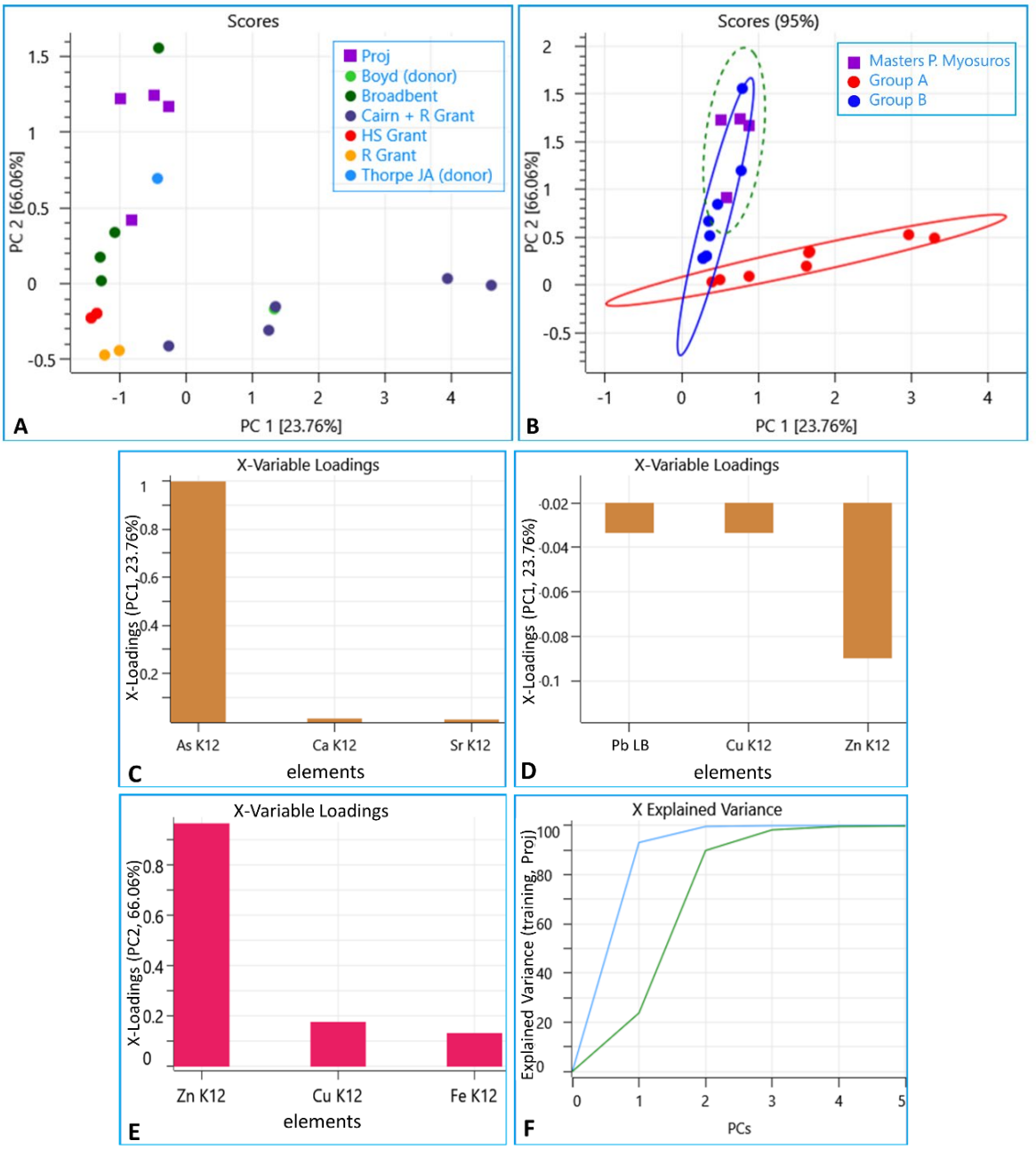

Fig. 5.10: The median of XRF data collected from *P. myosuros* were projected onto the PCA model created for the AM *Thylogale* spp. skins and first shown in Fig 5.2 (**A**). It shows that the *P. myosuros* data coincide with *Thylogale* spp. specimens identified as Group B (**B**). The *P. myosuros* data were predominantly described by PC2 (66.06% of variance), with Zn, Cu and Fe the main contributors to the loadings plot €. Negative values in PC1 also described the *P. myosuros* data (23.76% of variance), which was dominated by Zn, and Cu in loadings plot (**E**). (**F**) shows the explained variance within the *P. myosuros* data set.

5.3.6 A closer look at Group A

To investigate the characteristics of Group A beyond the As identified in Fig. 5.2, the data for Group A specimens, M.796, M.794, M.849, M.133, M.129 and M.128, were analysed in a separate PCA (scores plot Fig. 5.11). The explained variance plot, Fig. 5.11F, shows that this group of specimens is quite diverse with all five principal components needed to describe 90% of the data. Specimens with positive values in PC1 (M.494, M.794, M.128 and M.957) were described by Zn, Ca and S as seen in loadings plot, Fig. 5.11B. Zn in these specimens, is correlated with Cu (correlation loadings plot Fig. 5.11D), showing that these specimens were characterised by the same elements as those preserved in ethanol (Group B) although in lower concentrations. It was proposed that these specimens may have been exposed to ethanol for a short time before being reworked into dry skins, or that they were preserved initially in ethanol and later in a different preservative that, through leaching, reduced the concentration of Zn and Cu.

Specimens M.796, M.794, M.129 and M.133 were described by negative values in PC1 and distinguished by bromine (Br) as seen in loadings plot Fig. 5.11C. Br has recently been discovered to be a critical in the assembly of collagen IV, which is found at interface between derma and epidermal layers of the skin and thus may be an endogenous trace element in all the specimens studied (52,53). However, it is also an ingredient in the pesticide methyl bromide, which is known to have been used in the protection of museum specimens (35). Br is also readily available in salt (sodium chloride), especially sea salt, and may be indicative of the use of salt as a preservative or pickling agent (54,55). The evidence that Br was descriptive of just three of the specimens in Group A, suggests that its presence is related to preservative or pesticide use.

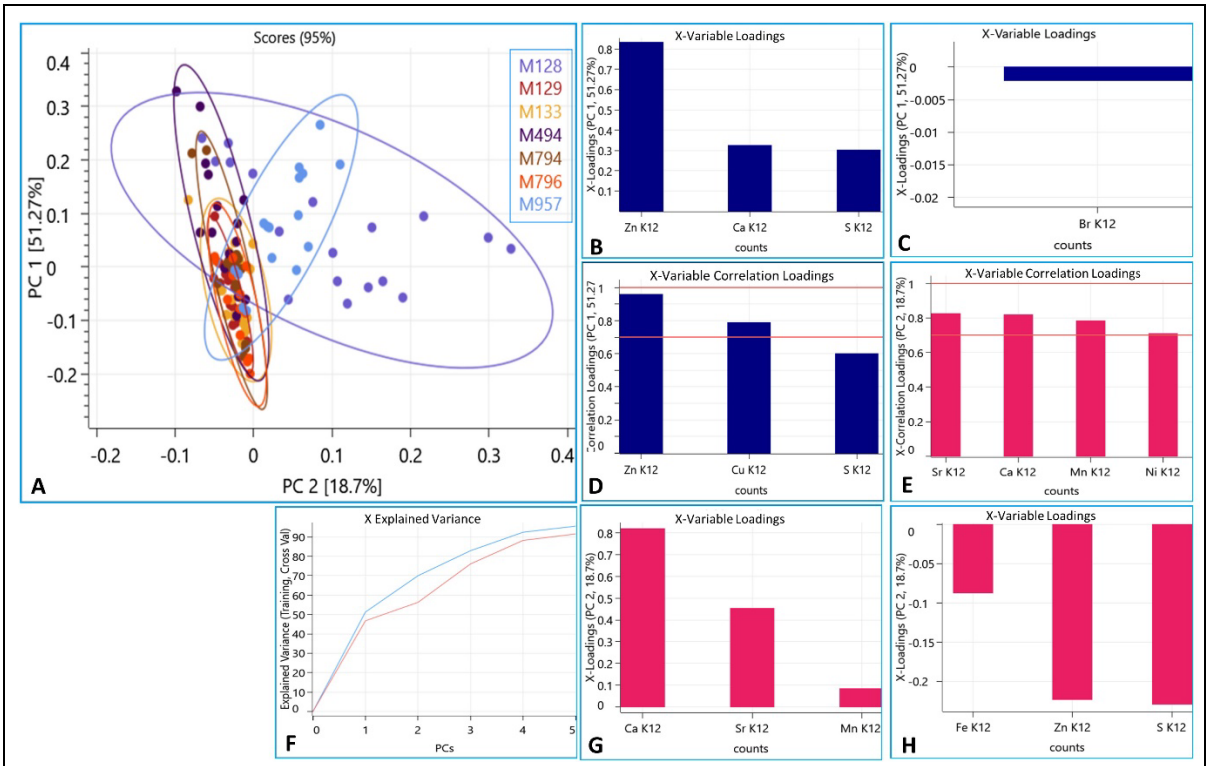

Fig. 5.11: PCA overview of XRF data collected from Group A with As removed from the analysis. Scores plot (**A)** shows all specimens described by PC1. Loadings plots (**B)** and (**C)** describe these specimens by Zn, Ca, S and Bromine (Br). In the specimens plotted above 0, Zn is correlated with Cu as seen in correlations loadings plot (**D)**. M.128 and M.957 are separated from the main cluster using PC2 and are described by Ca and Sr (loadings plot **G**) which are correlated with Mn and Ni in these two specimens (correlation loadings plot **E).** PC2 also contributes to the description of the main cluster as seen in loadings plot (**H)**. The explained variance plot, (**F)**, shows that this group of specimens is quite diverse with all 5 principal components needed to describe less than 90% of the data.

In this PCA, M128 and M957 were described by Ca, and Sr using PC2 (Fig. 5.10G) and these were correlated with Mn (Fig. 5.11E). All three elements are associated with bone which has been shown to be leached by unbuffered formalin and then deposited on soft tissues (56,57). In Chapter 4 (1), it was proposed that the presence of elements characteristic of bone, in specimens where little or no bone remains, is indicative of preservation and storage in

unbuffered formalin. To confirm that measurements taken from the specimen were not heavily influenced by remnant bone, the data for M.128 were cross-referenced with the notes identifying each site measured. This revealed that only three measurements were collected from sites where bone may have influenced results. Thus, the XRF data suggested that Group A is made up of specimens with diverse object histories and included some that are likely to have been preserved in formalin.

5.3.7 Using zinc concentration to re-establish object histories

Based on the high Zn concentrations shown using PCA results and the raw XRF data, it was deduced that specimens collected by Broadbent (A.5339, A.5340, A.5342, A.9725), H.S. Grant (M.2320, M.2321) and J.A. Thorpe (M.203) were initially preserved in spirits and later reworked into study skins (58). AM staff taxidermists began a program of reworking specimens in the museum collection as early as 1875 (6). In 1896-8, after the construction of a new spirit storehouse at the AM, a rationalisation of the spirit collection was undertaken in which "all marsupial skins were removed from the tanks and made up" (59). Using the same protocol, a history of preservation in alcohol can be inferred for two specimens attributed to "Goodman" in the MAMU collection (NHM.429 and NHM.430). While there is little information about the vendor of these specimens, Sir William Macleay notes the purchase of a group of "not particularised" mammals, birds and spirit specimens from "Goodman" in 1879 (60).

5.4 Conclusions

The object histories of early natural history specimens, including pesticide treatment and reworkings, are often unavailable due to data loss, or incomplete record keeping (28,35,61,62). Investigating the elemental residues of preservative chemicals from nineteen skins from the AM collection and six specimens from the MAMU collection, it was discovered that high concentrations of zinc are associated with a history of preservation in alcohol, or 'spirits'. The

ratio of zinc to sulfur was shown to have potential as a simplified method to determine a history of alcohol preservation.

Using this evidence, ten *Thylogale* spp. study skins were discovered to have been initially preserved in spirits and later reworked. This research has demonstrated the capacity to identify a history of alcohol preservation in both mounted and study skins. The study also compared data collected from two different taxonomic genera within the Class Mammalia, *Thylogale* and *Perameles.* The results of this protocol, provide strong evidence that the residues of preservation may be more broadly compared across zoological collections.

Another important aspect of this research is that preservation method has a significant impact on the success of DNA sequencing (63-66). Hence, this protocol has considerable value as a primary screening procedure to refine the selection of specimens for sampling. Additionally, alcohol preservation creates unique tolerances and vulnerabilities to storage environments, handling, and conservation treatments (67-70). This method to identify past preservation in alcohol can inform conservation decision-making and the long-term care of zoological specimens and collections (71). The capacity of this protocol to produce such information is likely to be highly valuable in connecting individual specimens with archival acquisition records. Application of this protocol to establish provenance for unprovenanced specimens based on mixtures of preservative residues is explored further in Chapter 6.

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Chapter 6

Matching Mystery Marsupials

6.1 Introduction

Throughout the previous Chapters, portable X-ray fluorescence (pXRF) has been applied in the investigation of remnant preservatives and pesticides to identify key elements and describe their relative concentrations within individual museum mammal specimens. Data processing using Compton normalisation has enabled comparison of elemental data collected from different specimens. Principal component analysis (PCA) has been applied to identify variance in the XRF data. The description of that variance has been used to define the subtle similarities and differences between specimens. Chapter 4, demonstrated that discrimination between specimens acquired by specific collectors was achievable using pXRF in combination with PCA (1). In Chapter 5, the protocol was used to identify histories of long-term alcohol preservation in groups of mammal specimens.

The application of the XRF/PCA protocol to historic museum specimens is further explored in this chapter. The first section describes the application of the XRF/PCA protocol to a study skins group comprised of two different species and collected by five field collectors. This experiment was designed to demonstrate that specimens continue to be differentiated according to their field collector when several collectors are included in the study group. The XRF data was then interrogated to identify key elemental characteristics that discriminated the work of each collector. Focus was then drawn to the practice of curator and field collector, George Masters (1837-1912). The elemental composition of specimens he collected was discussed with reference to his practice. Finally, XRF data collected from five ‘no data’ specimens from the Australian Museum, Sydney (AM) and six from the Macleay collection from the Chau Chak

Wing Museum at The University of Sydney (MAMU) were compared to data from specimens preserved by George Masters.

As in previous chapters, data points are plotted in the scores space based on their variance from the mean which creates clusters of data points that have similar variance. Because the XRF data is representative of both the elements and their relative concentrations, the data points that cluster together in scores space have similar elemental concentrations. Loadings plots describe the elemental variance that create clusters and therefore can be interpreted as characteristics of that cluster. All measurements collected from each specimen were marked on a scores plot to illustrate the compositional variation from each specimen and within each group. To enhance interpretation, the variable loadings plots have been ordered according to descending importance and split to show the components that contribute to the positive and negative values.

6.2 Experimental

6.2.1 Portable XRF Spectroscopy

Analysis was performed in air at the Australian Museum using a Bruker Tracer 5i portable XRF spectrometer (Karlsruhe, Germany), at 40 kV and 30 μA for 40 s with a Ti 25 Al 300 filter and using an 8 mm collimator. The Tracer was mounted on its stand beneath a custom-built specimen stage (detailed in Chapter 2, Section 2.3.1). The instrument was controlled via a laptop using Bruker ARTAX software (v8.0.0.476). As per requirements under NSW (Australia) state regulations, the operator was certified to use portable radiation emitting instruments.

The specimens were used directly without pre-treatment. Skins were positioned above the nose of the instrument and measurements were taken from a relatively flat surface which maximised direct contact to the instrument. Twenty sites were selected on each specimen for data

acquisition. At least two measurements were acquired from the belly, back, left and right sides, and the head. The tail was not measured as these were typically observed to be filled with coated wire. The specimen stage was cleaned with 70% ethanol and water, and a disposable paper towel, after the analysis of each specimen to minimise the cross-contamination of residues between specimens.

6.2.2 Data analysis

Spectra with dead times above 10%, or with poorly defined peaks, were excluded as they were considered unsuitable for reliable elemental analysis. ARTAX software (v7.8.2.0 Bruker) was used to conduct Bayesian deconvolution on the remaining XRF spectra, with escape, pileup peak, and background correction enabled for peak area evaluation. Compton peak area was calculated for each spectrum using *region of interest* in ARTAX. Results were exported to a Microsoft© Excel (.xlsx) format. Using Excel, the XRF peak area data were normalised to the Compton peak area (18.5-19.5 keV) for each spectrum. No further pre-processing was undertaken. The normalised data set was imported into VEKTOR DIREKTOR (v1.1 KAX Group, Australia) where PCA was performed using random cross-validation, and mean centring for 1000 iterations and five components.

6.2.3 Materials

Twenty-eight marsupial specimens were included in this study, representing two genera, *Thylogale* spp. and *Perameles myosuros* from the AM and MAMU collections. Specimens with known provenance were selected to represent the work of six field collectors between 1868 and 1913.

Table 6.1: *Thylogale* spp. (pademelon) specimens from the AM in this study.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number
K. Broadbent	1879	*T. billardierii* ♀	Tasmania	Study skin	A.5339
K. Broadbent	1879	*T. billardierii* ♂	Tasmania	Study skin	A.5340
K. Broadbent	1879	*T. billardierii* ♀	Tasmania	Study skin	A.5342
K. Broadbent	1880	*T. thetis* ♂	Darling Downs, QLD	Study skin	A.9725
R. Grant & E.J. Cairn	1888	*T. stigmatica* juvenile	Bellenden Ker, QLD	Study skin	M.128
R. Grant & E.J. Cairn	1888	*T. stigmatica* ♀	Bellenden Ker, QLD	Study skin	M.129
R. Grant & E.J. Cairn	1888	*T. stigmatica* ♂	Bellenden Ker, QLD	Study skin	M.133
R. Grant & E.J. Cairn	1889	*T. stigmatica* ♂	Herberton district, QLD	Study skin	M.494
R. Grant & E.J. Cairn	1889	*T. stigmatica* ♀	Herberton district, QLD	Mount	M.496
R. Grant	1892	*T. thetis* ♀	Bellinger River, NSW	Study skin	M.794
R. Grant	1892	*T. thetis* ♂	Bellinger River, NSW	Study skin	M.796
H.S. Grant	1913	*T. thetis* ♀	Barrington River, Bulliac NSW	Study skin	M.2320
H.S. Grant	1913	*T. thetis* ♂	Barrington River, Bulliac NSW	Study skin	M.2321
Unknown	Unknown	*T. stigmatica* ♀	Unknown	Mount	PA.999
Unknown	Unknown	*T. stigmatica* ♂	Unknown	Mount	PA.1002

Table 6.2: *Thylogale* spp. (pademelon) specimens from MAMU in this study.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number
Unknown	Unknown	*T. stigmatica* ♂	Herbert River, QLD	Study skin	NHM.408
Goodman	1879*	*T. stigmatica* ♀	Richmond River, NSW	Study skin	NHM.429
Goodman	1879*	*T. stigmatica* ♀	Richmond River, NSW	Mount	NHM.430
Goodman	1879*	*T. stigmatica* ♂	Richmond River, NSW	Mount	NHM.431
Unknown	Unknown	*T. billardierii* ♀	Tasmania	Mount	NHM.437

*This date represents the specimen entering the MAMU collection

Table 6.3: *Perameles myosuros* (bandicoot) specimens from AM and MAMU collections.

Field collector	Expedition Year	Species	Collection location (as labelled)	Form	Accession number	Museum
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.435	AM
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.436	AM
G. Masters	1868	*P. myosuros* ♂	Salt River, WA	Study skin	P.437	AM
G. Masters	1868	*P. myosuros* o	Salt River, WA	Study skin	P.440	AM
Unknown	Unknown	*P. myosuros* ♀	King Georges Sound, WA	Mount	NHM.436	MAMU
Unknown	Unknown	*P. myosuros* ♂*s*	Unknown	Study skin	M.2440	AM
Unknown	Unknown	*P. myosuros* o	Unknown	Mount	M.1121	AM
Unknown	Unknown	*P. myosuros* ♂	Unknown	Mount	M.1122	AM

6.3 Results and Discussion

6.3.1 Specimens continue to group according to field collector

Prys-Jones observed that the use of certain preservatives "appears to have been characteristic of particular collectors" (2 p.251) supporting the findings reported in Chapter 4 (1). In this section, the robustness of the XRF/PCA protocol was tested. XRF data from specimens studied in Chapter 4 was augmented with data collected from four *P. myosuros* (bandicoot) study skins collected by George Masters and two *Thylogale thetis* (pademelon) study skins collected by H.S. Grant (c.1892-1944).

PCA results, shown in Fig 6.1, confirmed that specimens obtained by the same collector clustered together based on their elemental composition. This finding supports the hypothesis that specimens acquired by the same field collector may be connected to each other via their elemental composition. The two pademelon specimens attributed to H.S. Grant clustered together in the PCA scores space (Fig. 6.1A). These specimens were described predominantly

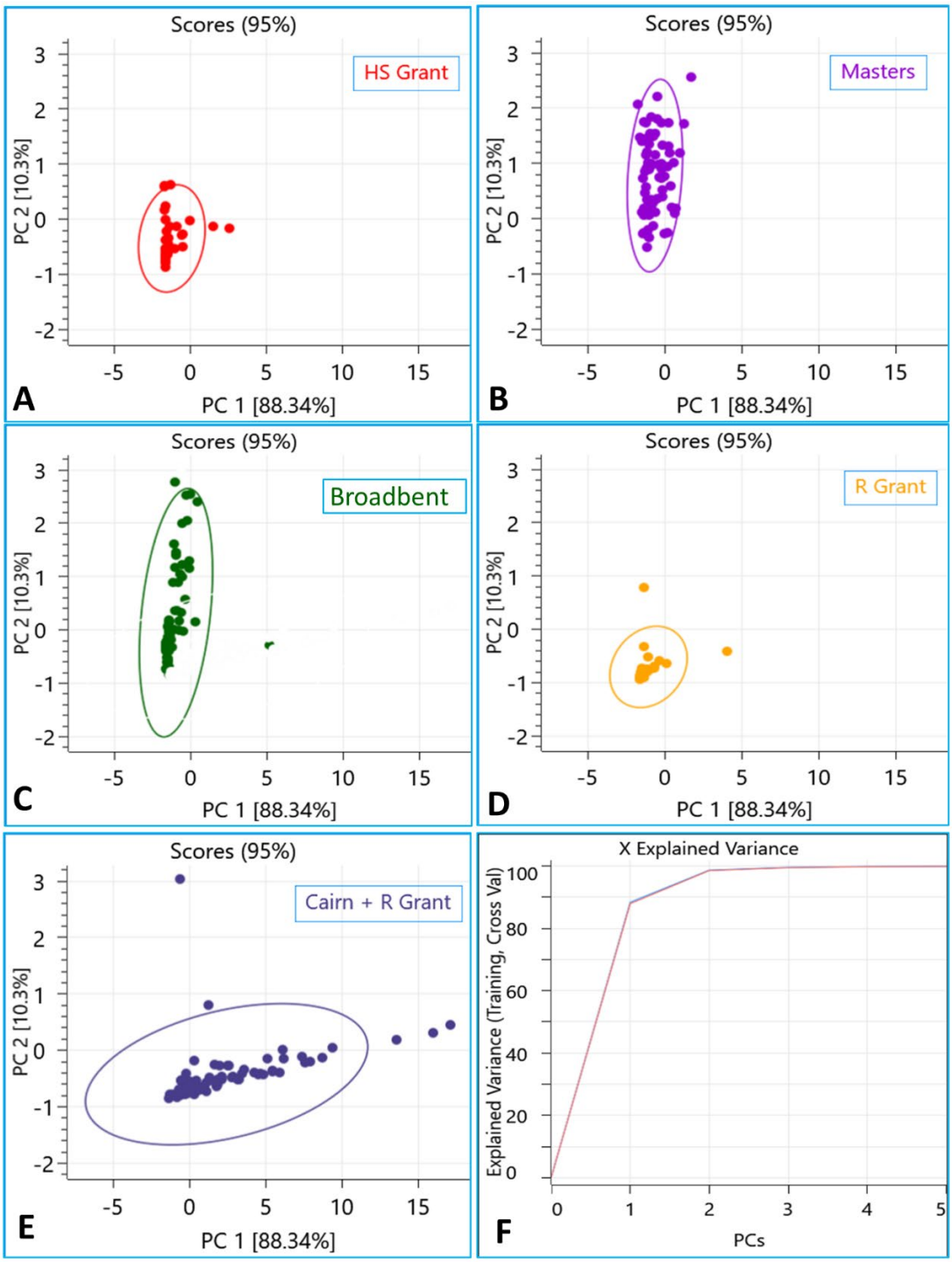

Fig 6.1: PCA of study skins from the AM showed that skins attributed to the same field collector, clustered together. Two pademelons attributed to H.S. Grant clustered together along PC2 (**A**) as did four study skins attributed to Masters (**B**). Four study skins attributed to Broadbent clustered along PC2 (**C**). Two study skins attributed to R. Grant also clustered together (**D**). Five study skins attributed to Cairn and R. Grant clustered along PC1(**E**). Over 98% of the variance across the data set was explained in the first two PCs (**F**).

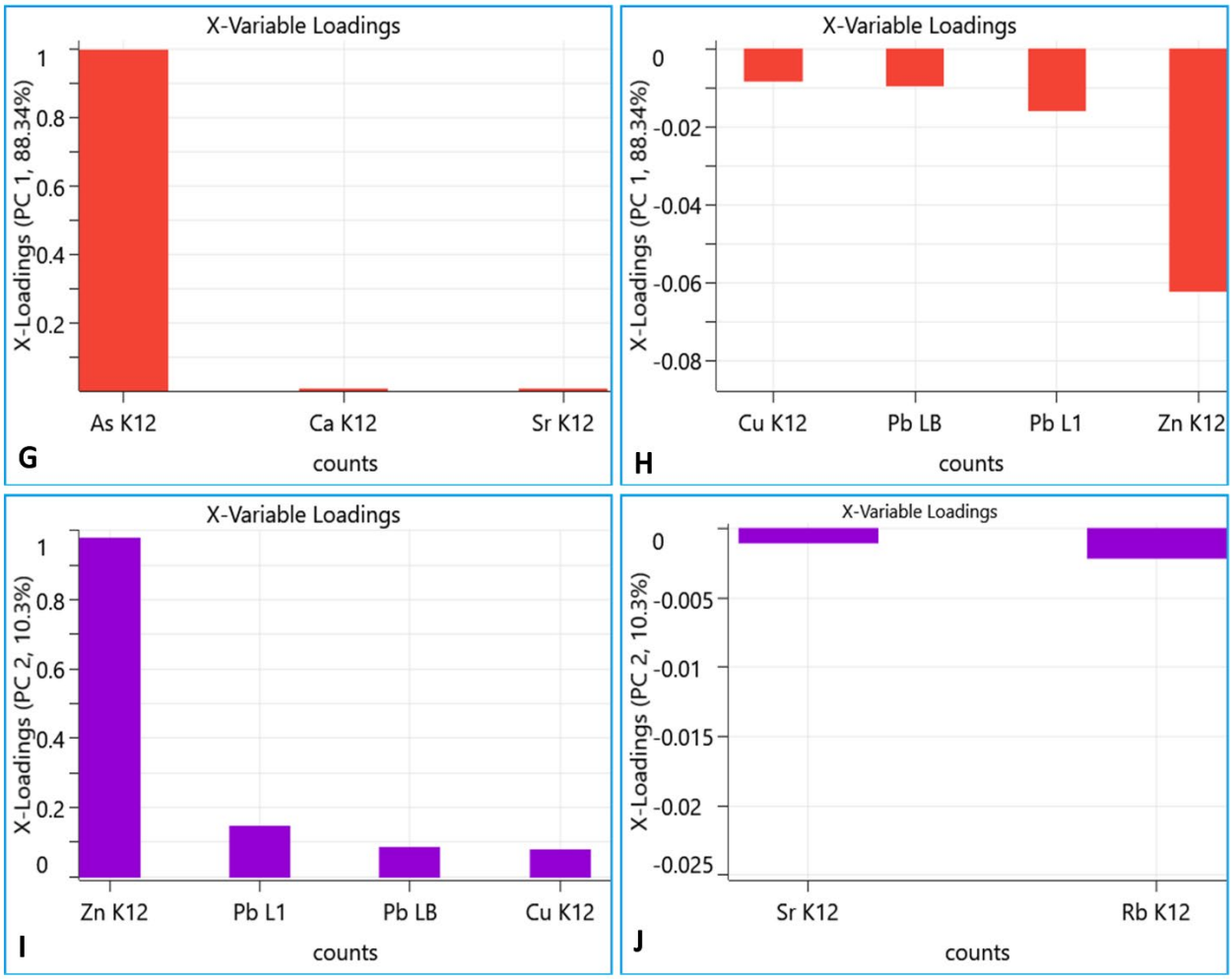

Fig 6.2: The variable loadings for the PCA shown in Fig 6.1. Loadings for PC1 are shown in (**G**) and (**H**). The variable loadings for PC2 are shown in (**I**) and (**J**).

by PC1 as contributions from Zn (Fig. 6.2I, J). The four bandicoot study skins collected by Masters formed their own cluster along PC2 and both the PC1 and PC2 loadings plots describing that variance are dominated by Zn (Fig. 6.1B, Fig. 6.2I, J). In agreement with the results described in Chapter 4, specimens collected by Broadbent (Fig. 6.1C), R. Grant (Fig. 6.1D), and Cairn working with R. Grant (Fig. 6.1E) clustered together. PCA showed that the specimens, overall, were very similar to each other with over 98% of variance across the entire data set explained in the first two PCs (Fig. 6.1F).

6.3.2 Investigating the discriminating elements

Additional interrogation of the PCA results was undertaken to identify the elemental characteristics that discriminated one field collectors practice from another. The results indicated that some field collectors used distinctive preservative mixtures. The spread of the data from specimens collected by Broadbent captured in PC3 was due mainly to Pb, Cu, Fe and Hg as seen in the loadings plots Fig. 6.3A, B. The bandicoot study skins collected by Masters (in purple) were described in PC3 by Zn (Fig. 6.3C) and in PC4 by Pb, K and Sr (Fig. 6.3D). The concentration of Zn, Cu, Fe and Hg in skins collected by Broadbent, and Zn and K in those collected by Masters, are important in discriminating between the two groups. Examination of the XRF counts data refined those differences. Bar plots in Fig. 6.4 present the median XRF counts produced by specimens collected by Broadbent and Masters (Fig. 6.4A and B, respectively). These show that high K and As concentrations were features of the Masters' specimens, while high concentrations of Hg, Fe and Cu coupled with relatively low As concentration discriminated the Broadbent specimens. High concentrations of Zn and Pb were common to the work of both collectors.

The presence of Pb, as a characteristic element in ethanol preserved specimens collected by both Broadbent and Masters, is in contrast with the spirit specimens collected 20 years later by R. Grant or H.S. Grant. There is little documented evidence of the use of Pb as a preservative in field collection manuals of the nineteenth century but there are several potential sources. Tubs lined with Pb for bathing specimens in water-based preservative solutions (3-5) and Pb labels may have contaminated preserving fluids (4,6,7). The preserving alcohol may have been the source of Pb. Although Pb was not commonly used as a preservative of wine and spirits made for human consumption at this time (8,9), spirits made in stills with a Pb head or cooling worm were contaminated with Pb (9,10). The residents of colonial Sydney enthusiastically

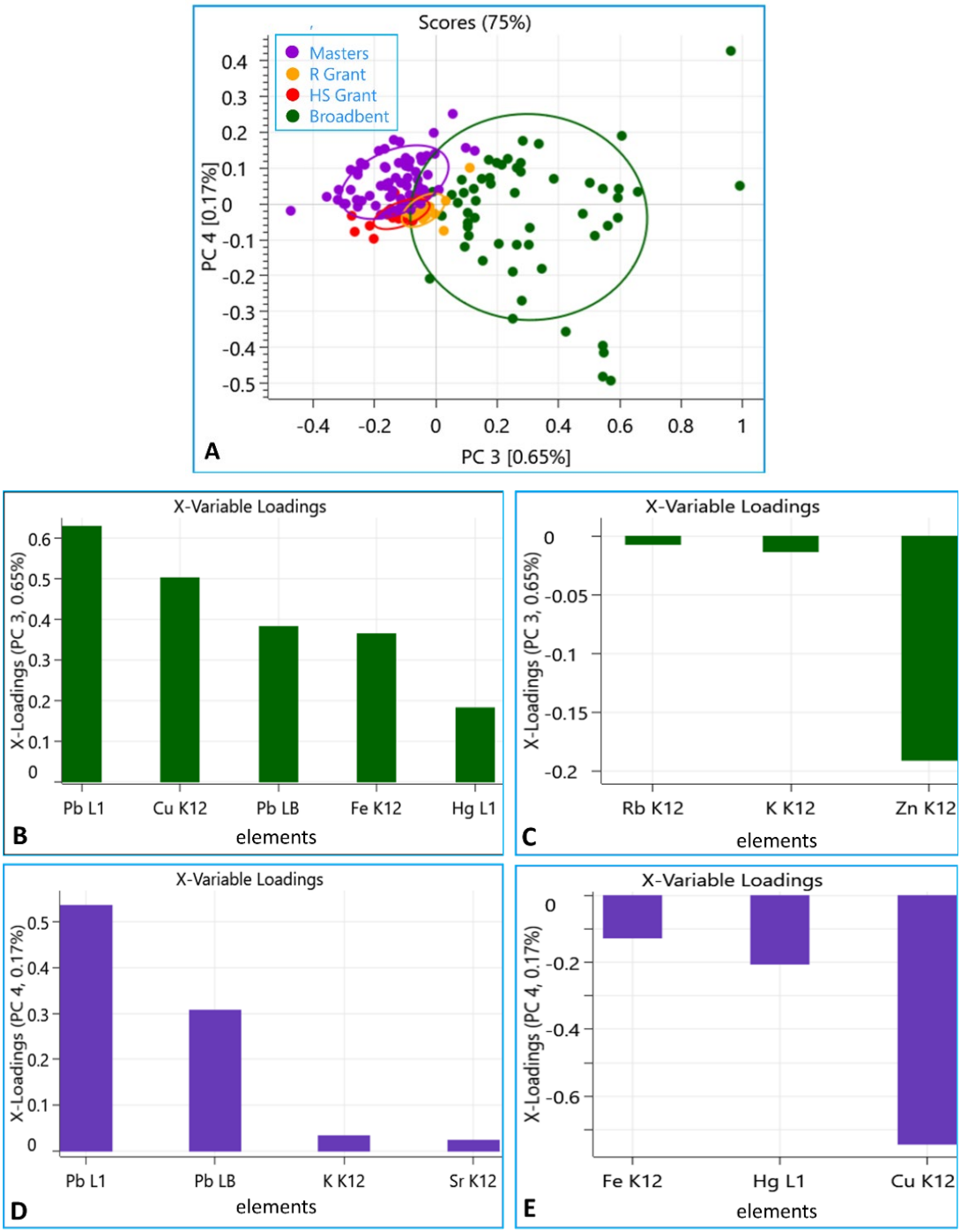

Fig 6.3: PCs 3 and PC4 show differences in the elemental composition of specimens collected by Masters and by Broadbent. Scores plot (**A**) shows that Broadbent specimens (in green) are discriminated from those collected by Masters by Pb, Cu, Fe and Hg (**B**). Masters' specimens were discriminated by Zn, K and Rb in PC3 (**C**) and Pb, K, and Sr in PC4 (**D**). The explained variance of each PC is small which indicated that these are subtle differences in the preservatives used by each collector.

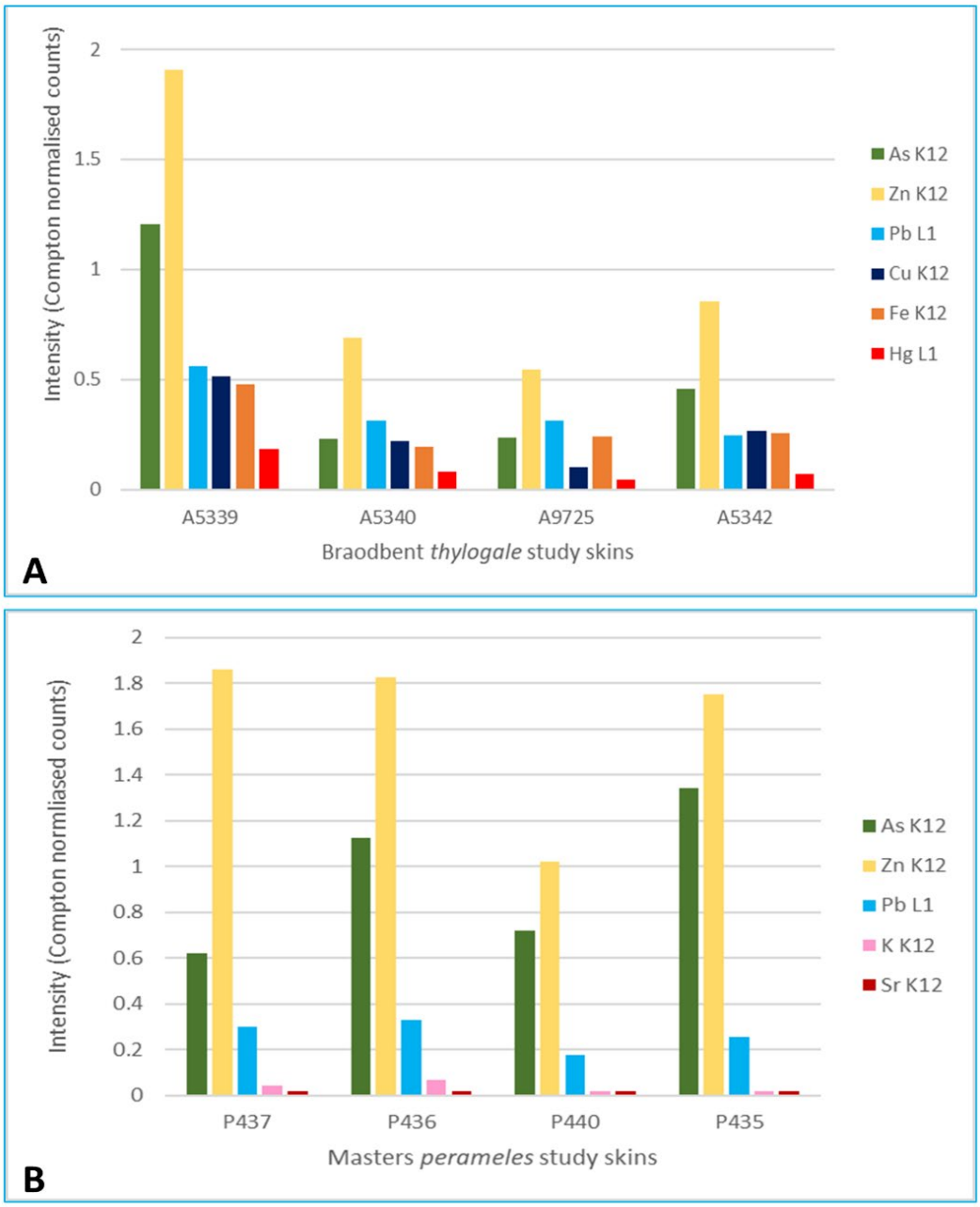

Fig. 6.4: Bar charts of median XRF counts for specimens collected by Broadbent (**A**) and Masters (**B**). High concentrations of K and As were discriminating features of the Masters' specimens. High concentrations of Hg, Fe and Cu coupled with relatively low concentrations of As discriminate the Broadbent specimens. Both sample sets were characterised by high concentrations of Zn and Pb.

produced spirits for medical purposes and consumption despite the regulation of distillation and the importation of stills (11,12). Hopkins reported that stills constructed from available materials included worms made from gun barrels likely soldered together with lead, pewter, or tin (13).

Alternatively, Pb may have been intentionally added to preserving spirits. Gerard Krefft noted (Fig. 6.5) the inclusion of Pb in spirits used for specimen preservation on the Philosophical Society of Victoria's expedition to the Murray and Darling Rivers (also known as the Blandowski expedition) of 1856-1857 (14 p.4). Lead acetate gained popularity after Thomas

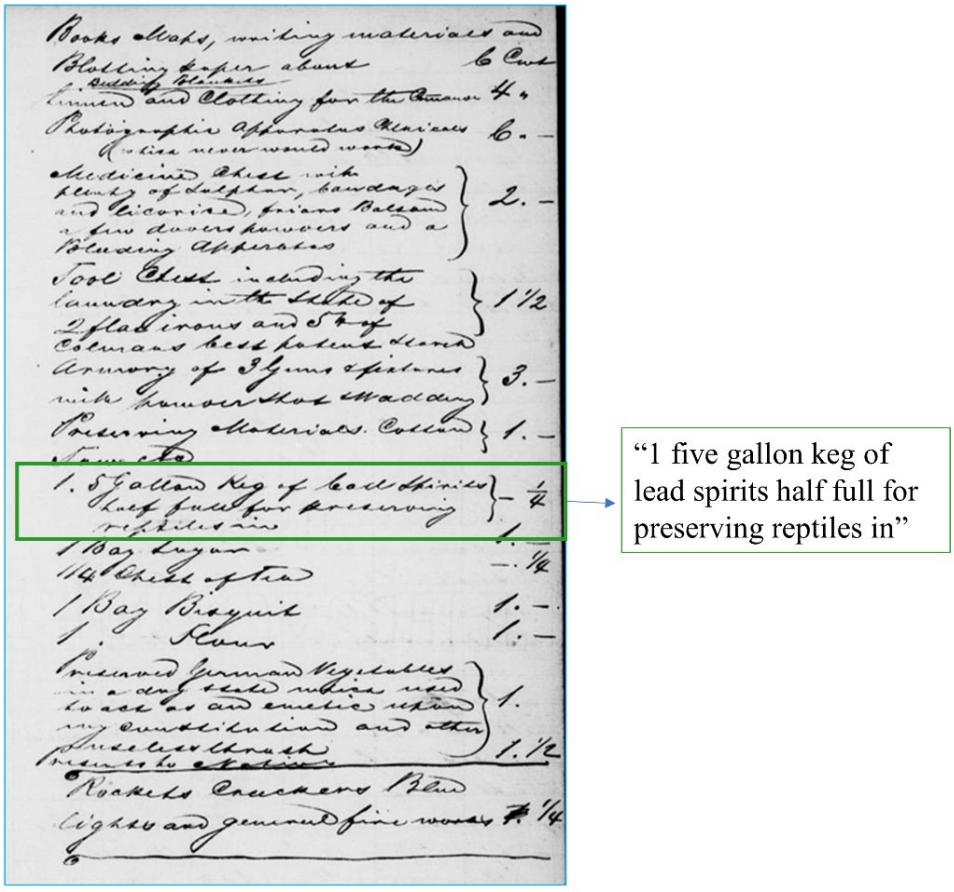

Fig. 6.5: Krefft's manuscript documenting the preparations for the Philosophical Society of Victoria's expedition to the Murray and Darling Rivers of 1856-1857 (also known as the Blandowski expedition) describe a keg of lead spirits for preserving specimens (14).

Goulard's treatise on its effects in 1784 (15). It was used in the medical and veterinary practices of the early nineteenth century as a disinfectant and 'astringent' for restriction of body fluids (16,17). Mixed with other ingredients including spirits, lead acetate was recommended for the treatment of skin conditions in animals and humans into the early twentieth century (18-21). Thus, Pb may have been considered useful in disinfecting skin specimens. Interestingly Bacon, *et al*. (22) and Pequignot (23) both found Pb in high concentrations in early zoological specimens but were unable to identify a cause for its presence. The presence of Pb in these older spirit specimens suggests that Pb may be related to wet specimen preservation and characteristic of specimens collected in the early to mid-nineteenth century.

A separate PCA was calculated to determine if it was possible to discriminate between specimens acquired by R. Grant from those collected by his son, H.S. Grant. Both collectors worked in the taxidermy department at the AM but at different periods, and both worked under the supervision of. J.A. Thorpe (24). The differences in composition of their specimens were identified in the scores plot in Fig. 6.6A. R. Grant's specimens were predominantly described by positive values in PC1 with loadings dominated by As, and smaller contributions from S, Ca, and Hg (Fig. 6.6B). H. S. Grant's specimens, described by negative values in PC1, were differentiated by Zn and Fe as shown in the loadings plot Fig. 6.6C. Based on the results described in Chapter 5, where high proportions of Zn were associated with long-term storage in ethanol, this finding indicates that the H.S. Grant specimens were held in ethanol for a longer time period than those collected by R. Grant.

6.3.3 Understanding the specimens collected by Masters

With the ambition of reuniting a 'no data' specimen with its contextual history, the bandicoots collected by George Masters in 1868 (first discussed in Chapter 5) were revisited to gain further insights into his preservative use. George Masters was curator and field collector for the AM

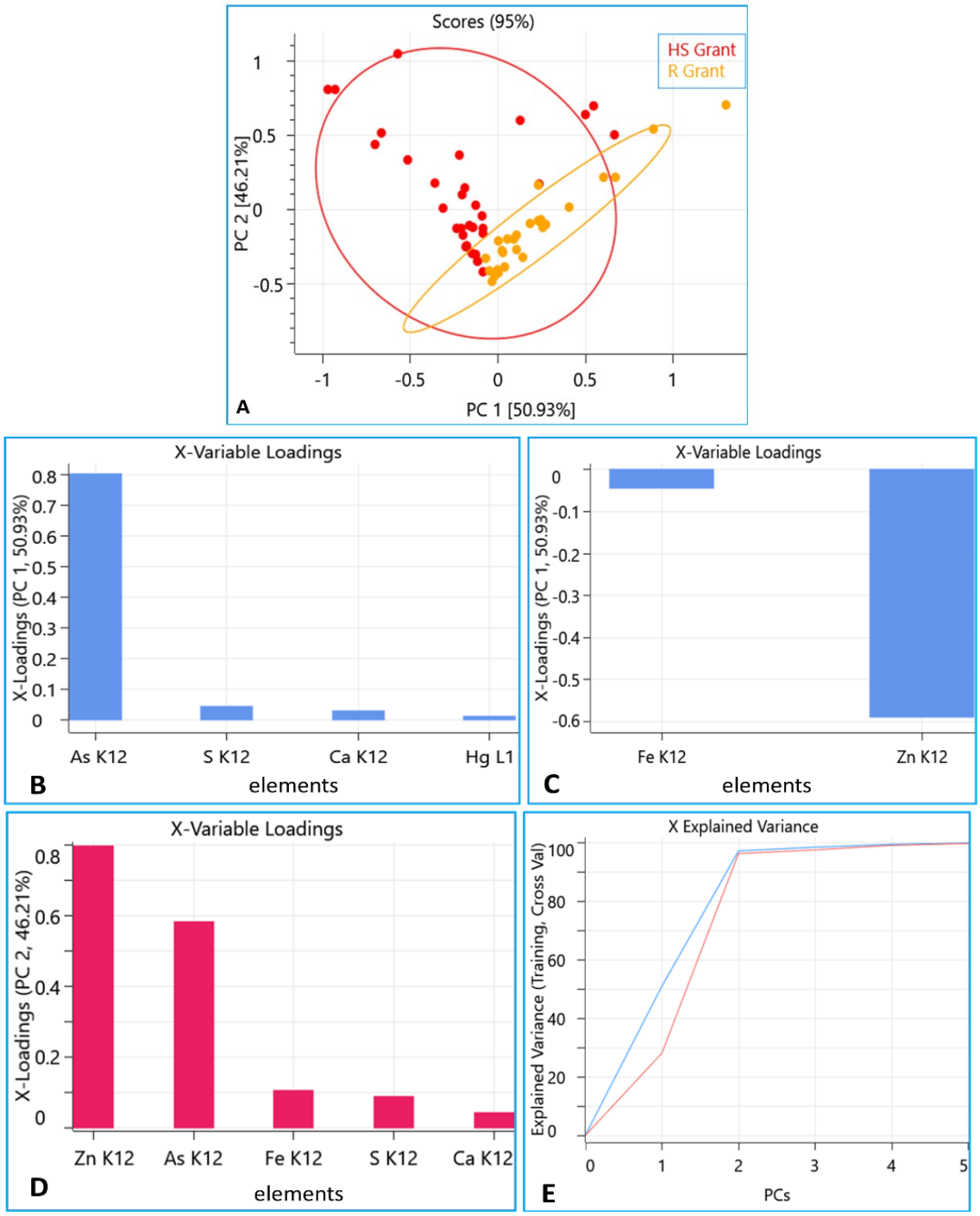

Fig 6.6: Discrimination between specimens collected by R. Grant and H.S. Grant using PCA (scores plot **A**). The variable loadings plot for PC1 (**B**) described the elements seen in specimens collected by R. Grant (As, S, Ca and Hg). Variable loadings plot for PC1(50.93%) (**C**) described the elements in specimens collected by H.S. Grant, Zn and Fe. Both groups were equally described by PC2 (46.21%) and the loadings plots shows Zn, As, Fe, A and Ca are important elements in the analysis (**D**). The explained variance plot shows 97% of the variance was described in these two PCs (**E**)

from 1864-1874 and "at one time more than half of the natural history specimens in the AM were of his taking" (25). According to the AM annual reports, he collected 734 mammals, 1163 reptiles, over 1281 birds, more than 15,000 insects, as well as fish and crustaceans for the Museum (26-33). Masters collected for the private collections of the Howitt family of Melbourne between 1856 and 1859, and Macleay family between 1860 and 1862 and again between 1874 and 1912 (34). It was hoped that using such a prolific collector as a reference, there was an increased likelihood of finding 'no data' specimens that matched the elemental composition of Masters' specimens.

Twenty-two bandicoots (*P. myosuros*) were collected by Masters at King Georges Sounds and Salt River, Western Australia in 1868 – twenty specimens were preserved in spirits and two as dry skins, according to the annual report of 1868 (31,34,35). At least three of these specimens were sent to the British Museum (now Natural History Museum, London) the following year (36) and are now known as specimens 1870.8.30.2, 1870.8.30.1, 1869.7.19.15. Fig 6.7 shows the letter of offer from Australian Museum curator, Gerard Krefft to John Gray, first keeper of zoology at the British Museum (now Natural History Museum, London). By 1879, nine of Masters' *P. myosuros* remained in the collection and were recorded in the AM catalogue – seven in spirits and two dry and mounted. It is unclear if the two mounted specimens recorded at this time (P432, P433) were those originally identified as dry skins in the annual report of 1868 (35). During the period between 1879 and 1912, seven of the Masters' *P. myosuros* specimens preserved in spirits were reworked into study skins. Paper tags, inscribed "no data 3/10/12", were attached to the hind legs of P.440 and P.435 which showed that at least two of the group had become temporarily disconnected from their contextual data (37). In 1984, the AM catalogue was updated to show only five of the original seven spirit specimens could be located. No updates were made for P.434 and P.439 which suggested they may also have become disassociated with their collection group and contextual data.

Four of the study skins known to have been preserved in spirits (specimens P.435, P.436, P.437, and P.440) and the two mounted skins (P.432, P.433) were analysed using pXRF and the data were interrogated using PCA. The analysis, in Fig. 6.8, showed that the elemental composition of the study skins was significantly different to that of the mounted skins. The spread of the data points for the study skins (in blue) were described by both PC1 and PC2 and, as previously shown in Section 6.3.2, Zn and Pb were again found to be important elements in their composition (Fig. 6.8A, C, F). The spread of data points for mounted specimens (in pink) were described by PC1 which is dominated by As and included Ca, Fe and Hg. In these specimens As, Hg and Ca were correlated (Fig. 6.8D).

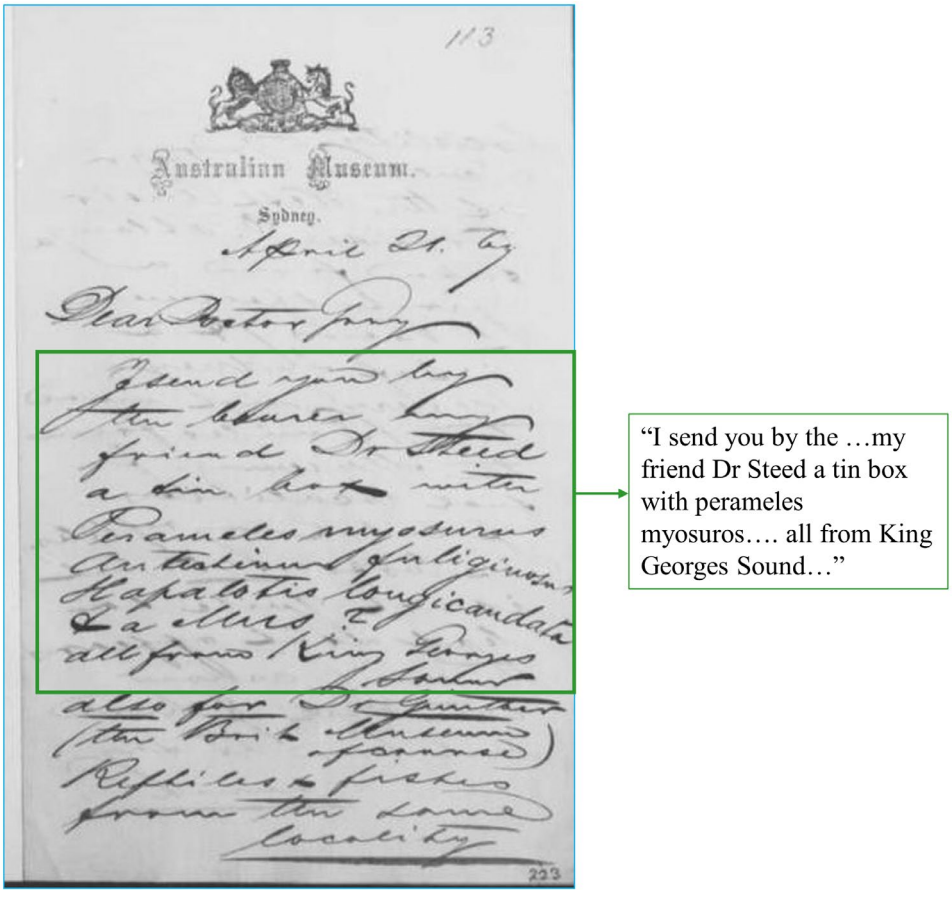

Fig. 6.7: As curator at the AM, Krefft sent this letter dated April 21st 1867, to the British Museum (now Natural History Museum London) offering bandicoot (*P. myosuros)* specimens (36).

The PCA was regenerated without the inclusion of data for As and the results are shown in Fig. 6.8. The bandicoots remained separated into two groups, each described by different elements (Fig. 6.A). Hendriksen identified an hierarchical decision-making process in field preservation where the first animals of a species captured would have been preserved in spirits (whole) and subsequent animals as dry skins (38). The significant differences in elemental composition observed in these six specimens indicated that this kind of decision-making may have been a part of Masters' field collection practice. The bandicoot study skins were described in PC1 predominantly using Zn and Pb contributions (Fig. 6.9C). In these specimens, Zn and Pb correlated in the PCA but this correlation was moderate ($R^2 = 0.49$) as shown in Fig. 6.9D, E. This provided further evidence that in these specimens, Pb was related to their preservation in alcohol. In the analysis of the mounted bandicoots, neither Zn nor Pb were identified as important elemental components in the distribution of data points. The spread of data for these specimens was described in PC1 by contributions from Ca, Hg and Fe (Fig.6.9B). The same elements were identified in the spread of data for these specimens in PC2, with Ca and Fe shown to be correlated (Fig. 6.9F, G). The elemental evidence suggests that Masters prepared the now mounted *P. myosuros* skins with a dry preservative possibly similar to that described by Richard Owen;

> "…if intended to be sent home dry, then the interior surface, with the bones, must be anointed with arsenical soap, and likewise the nostrils, ears, and lips, internally; and the hair or fur ought to be wetted with a weak solution of corrosive sublimate. The skin should then be stuffed with tow or cotton…" (7 p.379)

6.3.4 Comparing Masters' to 'no data' P. *myosuros* specimens

With this new information, the chemical composition of three 'no data' bandicoots (*P. myosuros)* were compared with those from Masters' bandicoots using the same protocol as the

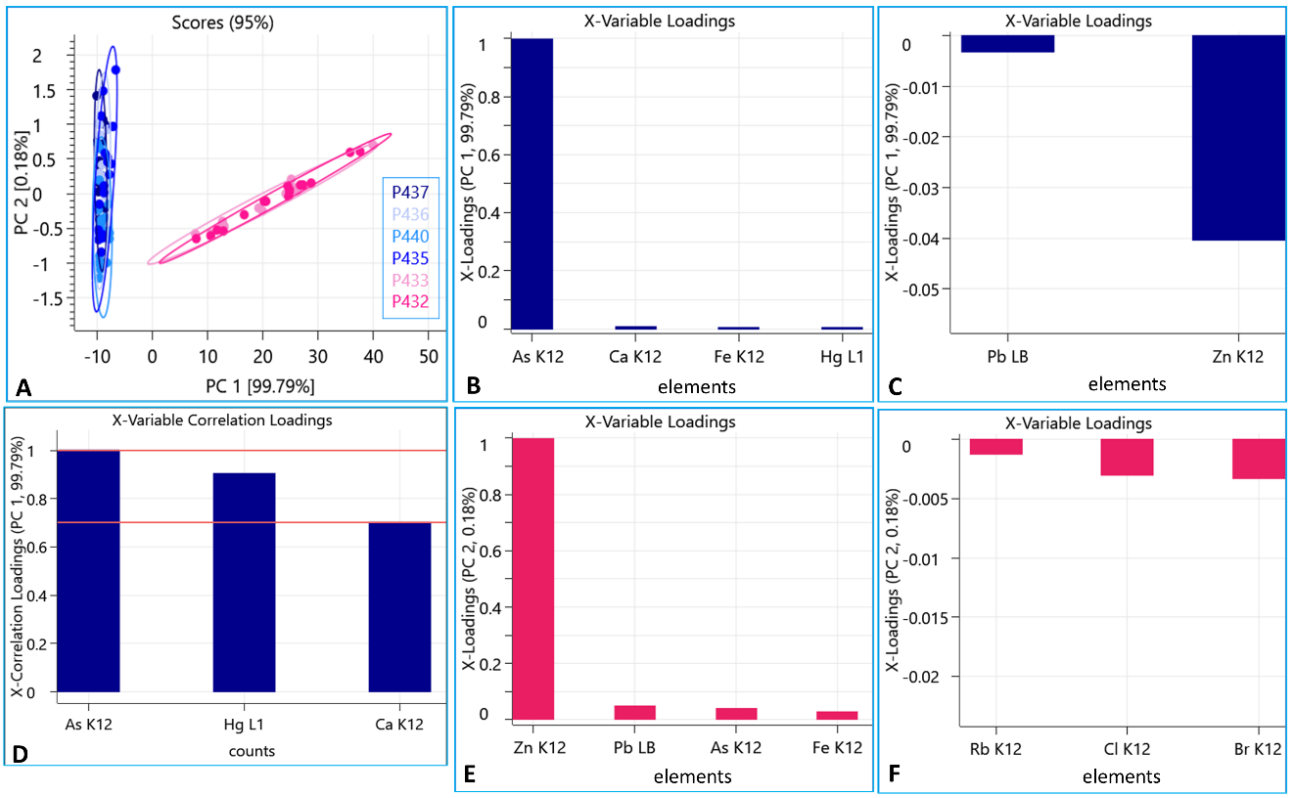

Fig. 6.8: PCA of bandicoots (*P. myosuros*) attributed to Masters. Scores plot (**A**) shows that the study skins (blue), once been preserved in spirits, clustered separately to the mounted specimens P.432 and P.433 (pink). The mounted specimens were described by PC1 in variable loadings plot (**B**). In these specimens, As, Hg and Ca were correlated (correlation loadings plot **D**). The study skins were described by PC1 (variable loadings plot **C**). Both clusters were described by PC2 (variable loadings plots **E** and **F**).

previous experiments. No attempt was made, prior to analysis, to ascertain provenance of these new specimens. AM staff retrieved these specimens from storage after searching for *Perameles* with no known data. The taxonomy of the three specimens was confirmed by mammalogist Dr Kenny Travouillon (39).

The median values for XRF measurements acquired from the 'no data' bandicoot specimens were first projected onto a PCA model created for the AM *Thylogale* spp. study skins and used in Chapter 5. This was undertaken to identify specimens with a history of preservation in ethanol (Group B) (Fig. 6.10A). The 'no data' bandicoots clustered closely to the Group B

specimens. The same PCA loadings groups that described *Thylogale* spp. Group B specimens (PC2) also described 82.4% of the variance within the XRF data collected from the 'no data' specimens (Fig. 6.10D). 6.8% of the variance within the 'no data' bandicoots were described by PC1 from Zn, Cu and Pb contributions (Fig. 6.10C). This comparison showed that, although now dry specimens, all three 'no data' *P. myosuros* were once preserved in ethanol.

The XRF data acquired from the 'no data' *P. myosuros* specimens was analysed together with bandicoot study skins collected by Masters using PCA. Fig. 6.11A showed that the XRF data acquired from the bandicoot study skins attributed to Masters specimens (in shades of blue) formed a cluster separate to the 'no data' specimens M.1121 and M.1122 (in greens). Scores plots (Fig. 6.11B, C) were coloured according to Pb and Fe concentration. They indicate that the relative concentrations of these two elements were key differentiators between the two groups. The spread of the data for M.1121 and M.1122 were described by PC1 using contributions from Zn which correlated with Pb (Fig. 6.11D, E), and by PC2 using contributions from Fe, Pb and Hg (Fig. 6.11G). The 'no data' bandicoot M.2440 data points, clustered tightly with the Masters' study skins, and was described predominantly by Zn and S contributions in PC2 (Fig. 6.11H).

The results prompted a search within the museum's archival records for additional documentation. Specimens M.1121 and M.1122 were registered on pages 61-2 in the AM's M register in 1896 with the same contextual details "perameles bouganvilii (♂), O.C. [abbreviation for Old Collection]" (40). That same year they were mounted. This record showed these specimens were collected prior to 1879 supporting the hypothesis made in Section 6.3.2 that Pb was connected to specimens collected from the field before 1885.

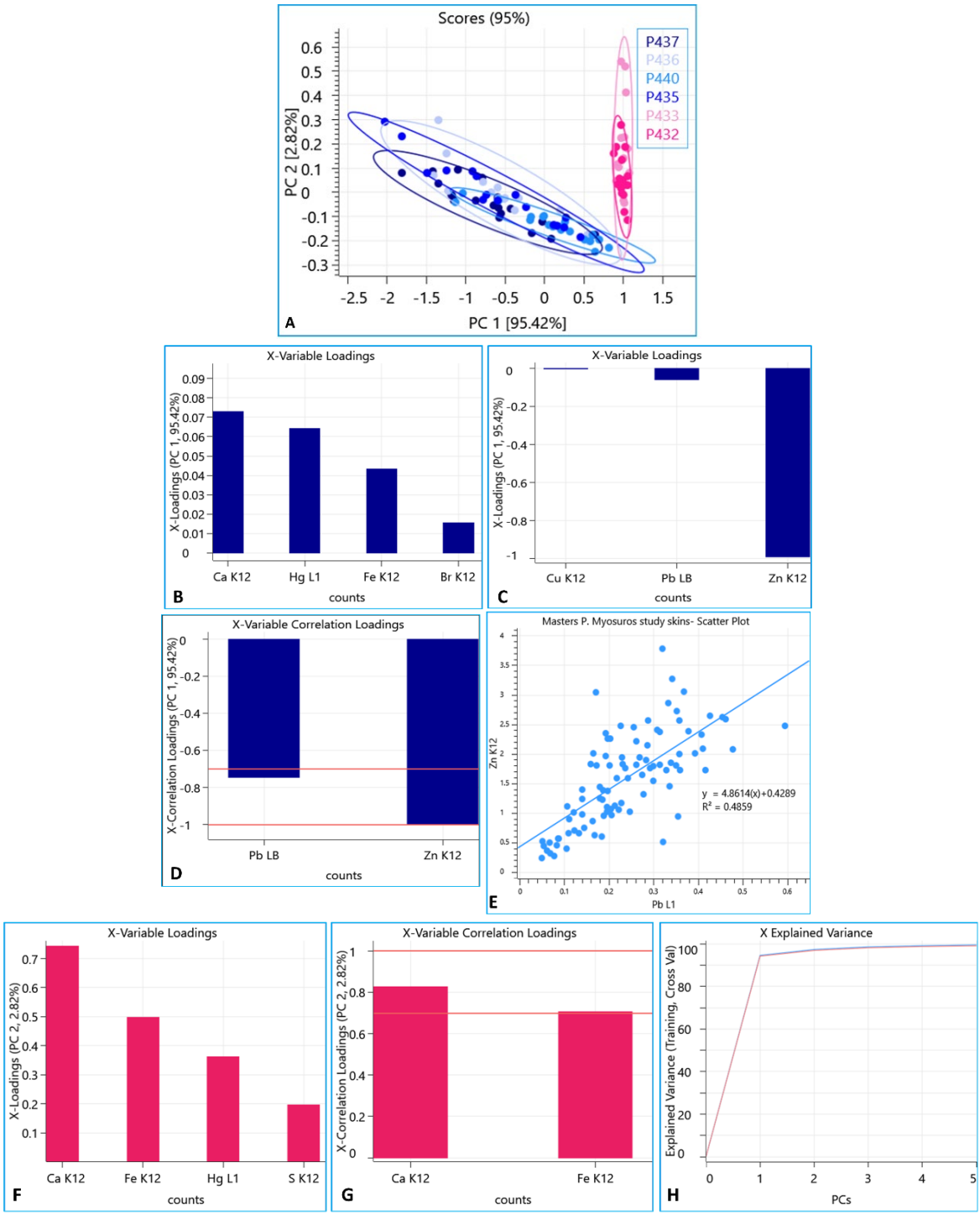

Fig. 6.9: Bandicoots attributed to Masters analysed by PCA without the inclusion of the As data. The scores plot (**A**) shows that mounted specimens clustered separately to study skins. PC1 variable loadings plot (**B**) described the spread of data points for mounted specimens P.432 and P.433. PC1 variable loadings plot (**C**) described study skins P.435, P.436, P.437 and P.440. Zn and Pb correlated in these specimens (correlation loadings plot **D**) and confirmed via Pearson's correlation (**E**). PC2 loadings plot (**F**) mostly described the mounted specimens where Ca and Fe were observed to be correlated (**H**).

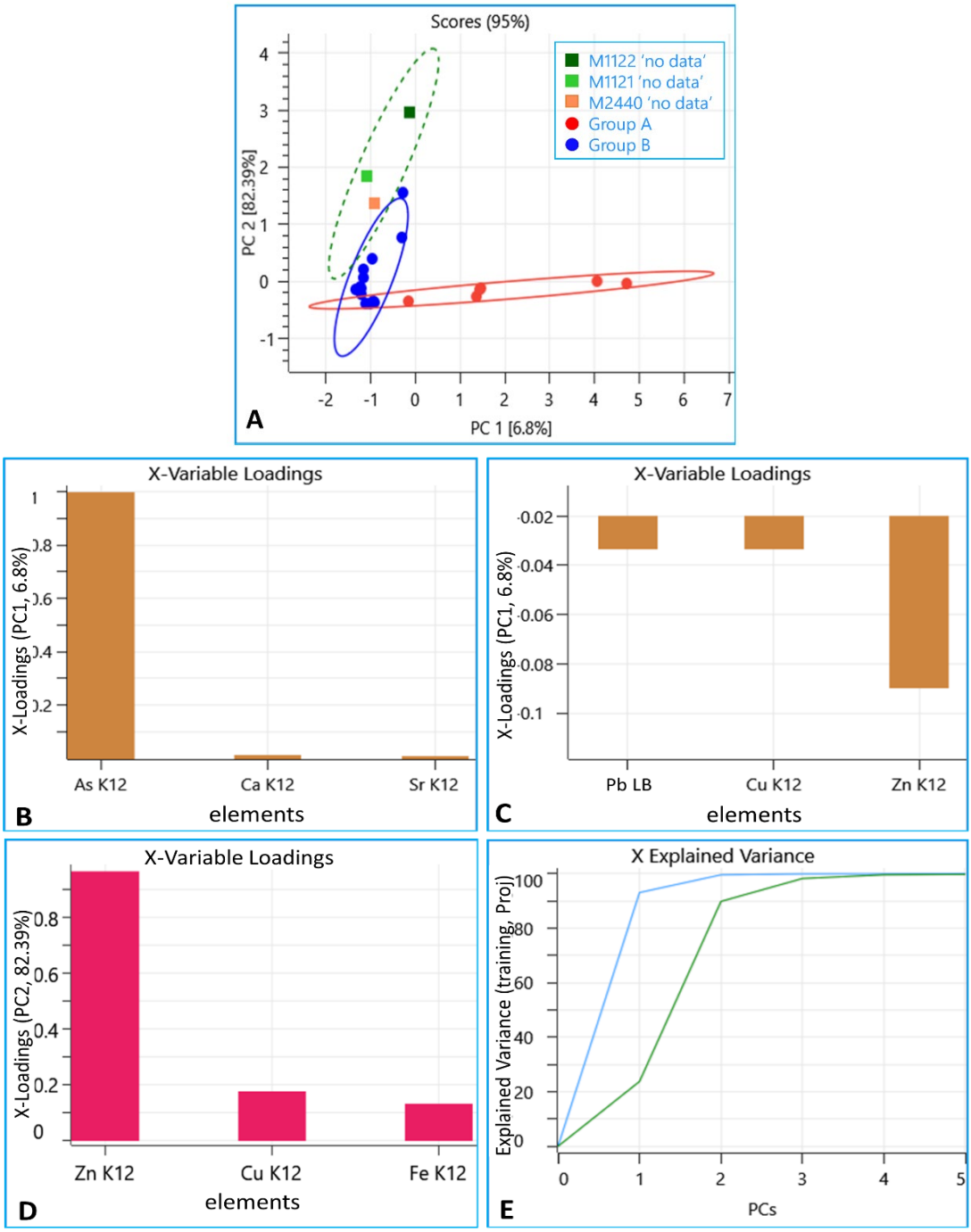

Fig. 6.10: Median values for the elemental data for the selected specimens were projected onto the model generated for *Thylogale* spp. study skins. Scores plot (**A**) showed these specimens were once preserved in ethanol. Using the same component groups that described the Group B specimens, the variance in data were described by PC1 (6.8%) (**C**) and by PC2 (82.39%) (**D**). Specimens without a history of ethanol preservation (Group A) were exclusively described by PC1 (**B**). Three PCs were required to describe the variance between the three 'no data' specimens in the variance plot (**H**).

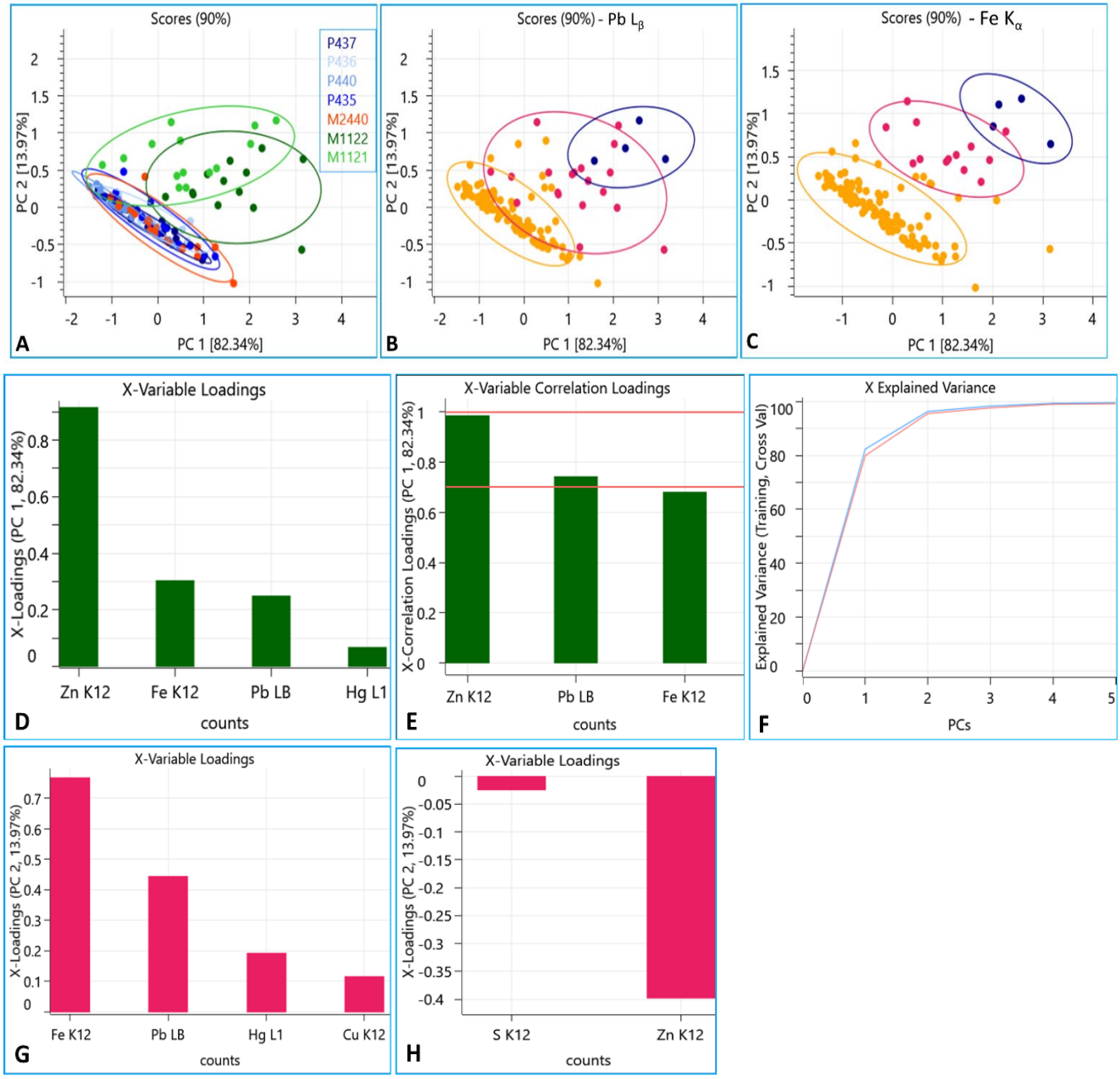

Fig 6.11: Data from the bandicoot study skins attributed to Masters P.435, P.436, P.437, and P.440 were compared with the data from the 'no data' bandicoots M.2240, M.1121, and M.1122. Scores plot (**A**) showed specimen M.2440 clustered tightly with the study skins attributed to Masters. Specimens M.1121 and M.1122 were discriminated by higher relative concentrations of Pb (**B**) and Fe (**C**) and were described by PC1 (82.34%) (**D**) in which Zn, Pb and Fe were correlated (**E**). PC2 (13.97%) also contributed to the description of the data from M.1121, and M.1122 (**G**). M2440 and the variance in the Masters study skins were described by PC2 (**H**). The explained variance plot (**F**) showed PC1 and PC2 were the dominant descriptors.

M.2440 bore a paper tag inscribed ‘no data’ identified and dated 1912. It was entered into the M register the following year on page 145-6 with the contextual notes “Perameles ?, (♀), No data, Old Coll.” (40). The details “bougainville” and description were added in pencil at a later date. The specimens’ similar elemental composition, appearance, and limb arrangement led to a review of the contextual data for all the *P. myosuros* bandicoots attributed to Masters (see Fig 6.12 below). Masters’ specimens P.434 and P.439 were last identified in 1879/80 where they were registered as specimens in spirits (41). It is possible that P.434 and P.439 may have been reworked at the same time as P.435, P436, P.437 and P.440 but had become dissociated from the other Masters’ *P. myosuros* bandicoots. This elemental analysis provided strong evidence that M.2440 was one of these missing Masters specimens.

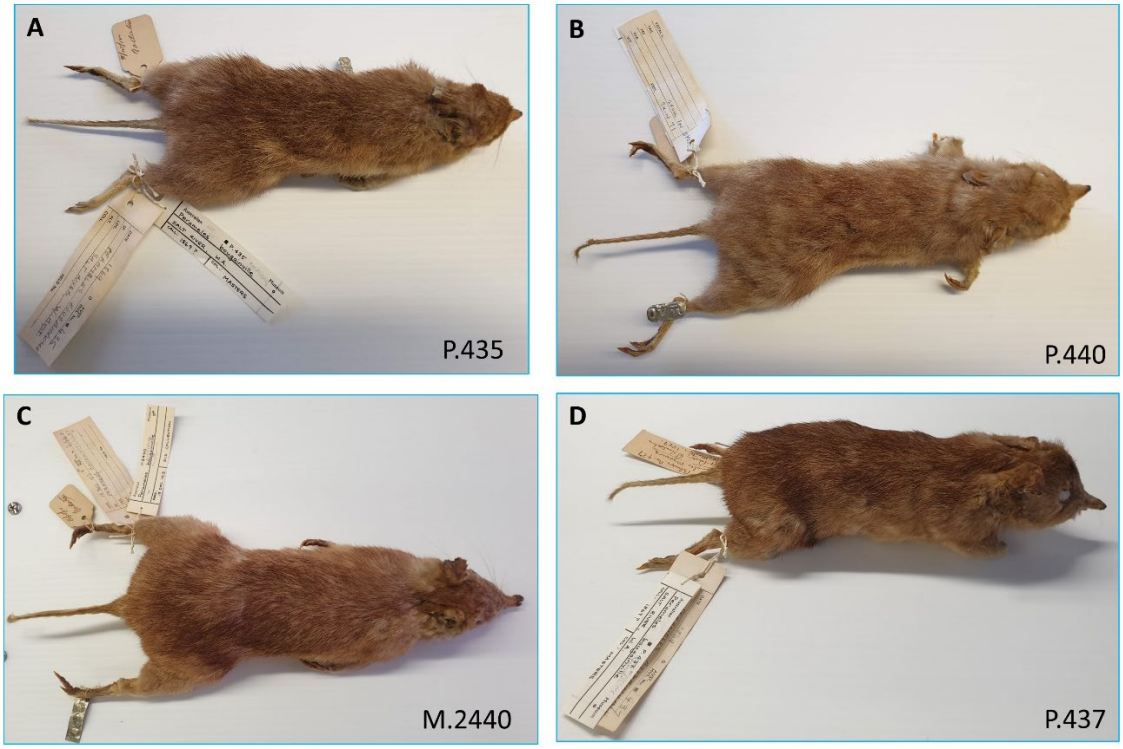

Fig. 6.12: The Masters’ bandicoot study skins P.435, P.440 and P.437 are pictured in (**A**), (**B**) and (**D**). These specimens were similar in elemental composition, physical appearance, and limb arrangement to M.2440 (**C**).

6.3.5 Mystery Macropus and Masters

Buoyed by the success of the re-attribution of M.2440, the protocol was applied to two 'no data' mounted *T. stigmatica* specimens held in the AM collection (PA.999 and PA.1002, Fig. 6.13). The unknown provenance of these specimens had long been considered problematic. These two mounted pademelons were described in the AM's Palmer (P) register at around 1879 as 'Halmaturus wilcoxi…N.S.W.', and a stamp indicating they had been on display in the museum's gallery. Both pademelons had remnant labels consistent with those used in early gallery displays. These provided the scientific name "*Macropus wilcoxi,* McCoy" and region 'Wide Bay Queensland'. Both specimens were stuffed with similar construction materials and techniques.

Fig. 6.13: Two pademelon (*Thylogale stigmatica wilcoxi*) without data were identified in the AM collection and pictured here. (**A**) PA.999. (**B**) PA.1002.

Median XRF data for PA.999 and PA.1002 were projected into a PCA model developed from *Thylogale* spp. study skins, and the results are presented in Fig. 6.14. Neither specimen had an elemental composition that reflected a history of long-term preservation in alcohol, as observed in Group B specimens (Fig. 6.14A) which were described by high relative concentration of Zn

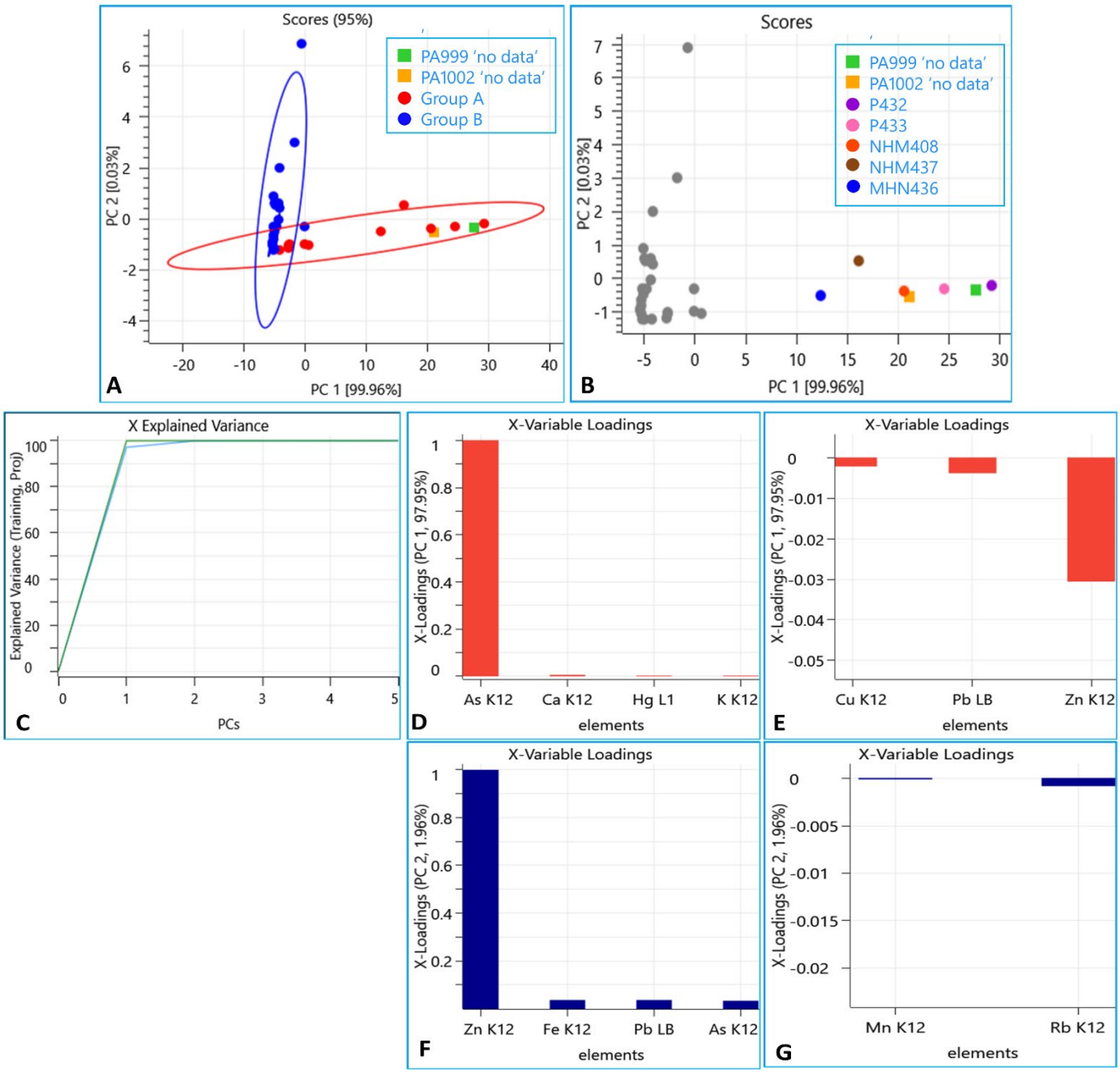

Fig. 6.14: Median XRF data for 'no data' pademelons PA.999 and PA.1002 were projected onto the model developed for *Thylogale* spp. study skins. They were found to cluster with Group A specimens (**A**) and the variance was described by PC1 (97.95%) (**D**). These specimens clustered with the two mounted bandicoots collected by Masters P.432 and P.433, and with three further 'no data' specimens from the MAMU collection, NHM408, NHM437 and NHM436 (**D**). The variance within these specimens was explained predominantly by PC1 (**C**).

in loadings plots Fig. 6.14E and F. Instead, 99.96% of the variance within the data for PA.999 and PA.1002 was explained by PC1. The loadings plot showed contributions from high concentrations of As, Ca, Hg and K (Fig. 6.14D). Both specimens plotted closely with the two mounted bandicoots collected by Masters (P.432 and P.433) shown in Fig. 6.14B. Three other specimens were included in this cluster, pademelon (*T. stigmatica*) NHM408, pademelon (*T. billardierii*) NHM437 and bandicoot (*P. myosuros*) NHM436. All were 'no data' from the MAMU collection.

The relationship between the Masters mounted bandicoots and the 'no data' specimens was further explored using PCA including only P.433, P. 432, PA.999, PA.1002, NHM408, NHM437 and NHM436. Elemental data for As was removed from the calculation, as it dominated the analysis and disguised subtle differences within the data set. Multiple measurements were used for each specimen to develop a representative overview of the variation in residues acquired over each specimen's history. The scores plot in Fig. 6.15A showed that PC1 described all the specimens from MAMU (NHM408, NHM437 and NHM436), and this made up 69.5% of the variance in the data set. Zn concentrations had the greatest contribution to PC1 (Fig. 6.15C) and this was interpreted as indicative of a period of storage in ethanol. The high Zn concentrations were especially notable in the pademelon *(T. billardierii)* NHM437. Similarity between the mounted Masters' bandicoots (P.432, P.433) and the 'no data' pademelons from the AM, PA.999 and PA.1002, were shown in this plot and the variance was described in PC1 as contributions from Fe, Ca, Hg and Pb (Fig. 6.15B). PC2 dominated the grouping of Masters mounted bandicoots (P.432, P.433), PA.999, PA.1002 and NHM437 with contributions from Ca, Fe, and Hg (Fig. 6.15E). NHM436 and NHM406 were discriminated from the other specimens by their levels of K, Pb and Rb content as described in

PC2 loadings (Fig 6.15F). The data from this diverse group of specimens required a minimum of four PCs to adequately explain the total variance (Fig.6.15D).

The similarity between the mounted Masters' bandicoots (P.432, P.433) and the 'no data' pademelons from the AM (PA.999 and PA.1002) warranted a search of documentary resources to support the association of Masters with these specimens. The subspecies, now known as *Thylogale stigmatica wilcoxi,* was first named as a full species *Halmaturus wilcoxi* by McCoy in 1866 (42). Thus, the earliest date for the collection of these 'no data' specimens is likely to be around this time. The entry into the AM's P register in 1879 provided the latest possible date for field collection (41). This evidence showed the time window for the collection of the specimens was between c.1866 and 1879. The location, 'Wide Bay, Queensland', found on the old display labels, matched two locations: a south facing bay near Cairns in tropical North Queensland, or a township 250 km north of Brisbane, in southern Queensland.

While the AM Annual reports show that Masters collected in the area of Ipswich and Pine Mountain, just west of Brisbane, during 1865 (28), the reports show no connection between 'Wide Bay, Queensland' and Masters. Whitley notes that Masters collected at Wide Bay, Queensland in 1865 (September to November) and 1867 (November) but provided no further details on the location, nor if the expedition was undertaken in connection with the AM (34). Masters, as Whitley explains, collected for himself and for William Macleay throughout his career. Therefore, a stronger connection between Wide Bay and the AM and Masters was considered desirable.

A search of historic newspaper and journal articles was undertaken via Trove (an online database of digitised Australian collections hosted by the National Library of Australia) using search terms including "collecting", "Wide Bay", and "Sydney Museum" (the name of the

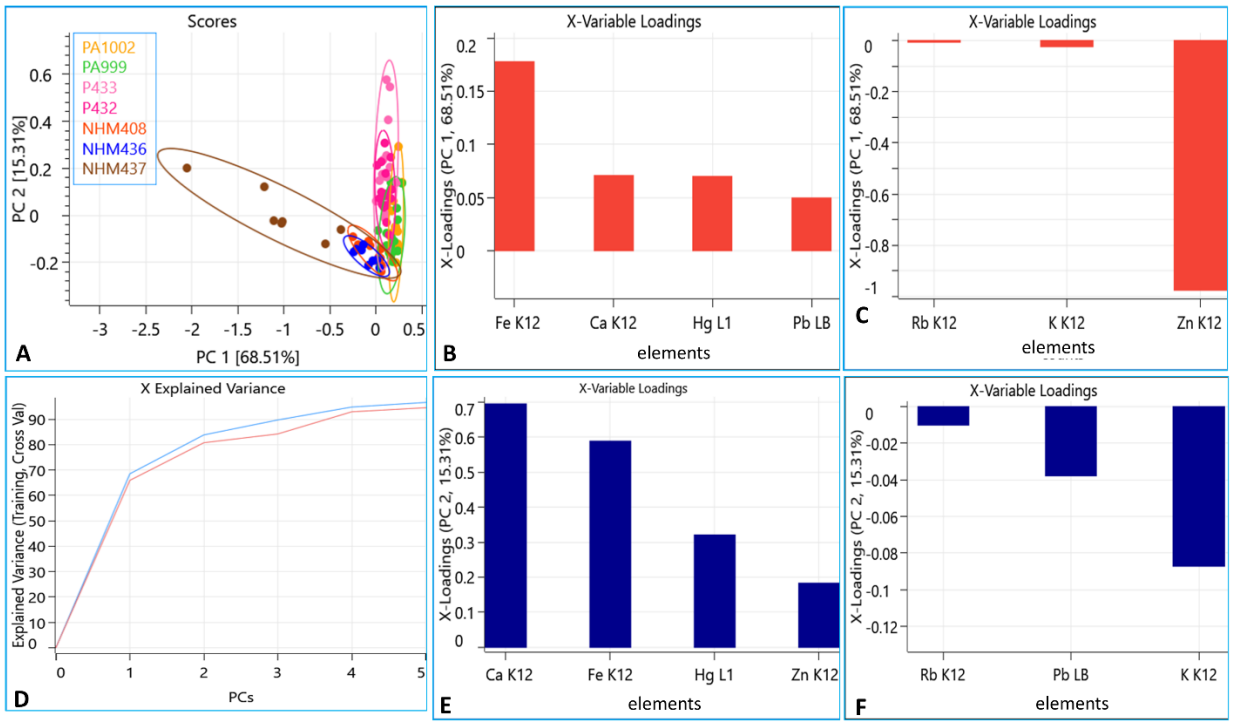

Fig. 6.15: Results of a PCA model including only specimens P.433, P. 432, PA.999, PA.1002, NHM408, NHM437 and NHM436, and with elemental As data removed. Scores plot (**A**) shows that 'no data' specimens from MAMU, NHM408, NHM437 and NHM436, occupy the space described by both PC1 (**C**) and PC2 (**E**), while the mounted bandicoots attributed to Masters (P.433, P. 432) and the 'no data' pademelons (PA.999 and PA.1002) clustered together. The variance of this cluster was predominantly described by PC2 (**E**). The 'no data' specimens NHM408 and NHM436 were described by negative contributions for PC2 (**F**).

Australian Museum at the time). The search yielded an article in the Maryborough Chronicle, Wide Bay and Burnett Advertiser dated 18 Dec 1867 (43 p.2) which reported;

> "Through the courtesy of Mr. George Masters, a gentleman at present sojourning at Maryborough in the capacity of collector of specimens of natural history to the Sydney Museum, we have had an opportunity of inspecting a recent instalment of his rich and varied collection, illustrative of the Fauna of the Wide Bay district, consisting of birds,

> insects, and a few reptiles and mammalia… An earlier series, even more comprehensive than the present one, has been already forwarded to Sydney…"(43).

The author went on to write;

> "Among the Mammalia we noticed a species of wallaby, only recently discovered and considered scarce. It is distinguishable from the better known [sic] kinds by the superior silkiness of its' fur" (43).

A further comment in the article suggests that few other field collectors operated in the area…

> "We have no doubt that the results of Mr Masters' labors [sic] will have the effect of attracting other scientific explorers to a field as yet but little touched by naturalists"(43).

A second and more detailed search of the AM archives produced an '1867 exchange list' which listed specimen/s of "Halamaturus wilcoxi, Wide Bay No. 4" amongst the mammals collected by Masters at Wide Bay (44). This documentary evidence, together with the analytical data, provides strong support for the hypothesis that specimens PA999 and PA1002 were collected by Masters at Wide Bay during 1867.

6.4 Conclusion

This is the first definitive application of pXRF combined with PCA to identify characteristic elements (and relative proportions of those elements) associated with each collector in this study. Older spirit preserved specimens and their collectors were characterised by the presence of Pb as well as Zn, and this is likely to prove to be a definitive attribute to characterise early Australian spirit preservation. Within the group of these older spirit specimens, the presence of Cu, Hg and Fe in Broadbent's specimens discriminated them from those prepared by Masters. The very similar work of R. Grant and H.S. Grant was differentiated by the relative concentrations of Zn and Fe, which were higher in the specimens prepared by H.S. Grant and indicated these specimens were stored in ethanol for a longer period.

The elemental composition of three ‘no data’ specimens were connected to George Masters. This new information redirected an archival investigation that revealed strong documentary evidence that both supported the connection and provided contextual data on field collection of each specimen. The results within this chapter demonstrate that the breakthrough protocol developed and discussed throughout this dissertation can bridge gaps in archival records resulting in the reconnection of ‘no data’ specimens with their field collection history.

Attention was focused on the practice of George Masters, and his use of both preservation in spirits and dry preservative preparations were recognised. XRF data acquired from ‘no data’ specimens were statistically compared to that of Masters using multivariate analysis and remarkable similarities between several specimens were revealed. These observations lead to the reattribution of provenance of one specimen of the now extinct bandicoot species, *P. myosuros*. The expansion of specimens with attributed provenance from extinct species is particularly important for research purposes. The elemental similarity between the work of Masters and two ‘no data’ pademelon (*T. stigmatica*) specimens prompted an in-depth document search, which supported the provenance indicated through the XRF analysis. The archives also revealed that Masters hosted exhibitions of his mounted specimens during his expedition in Queensland in 1867. Masters made a significant contribution to the wider body of Australian specimens in museums around the world, and information about his practice is likely to be beneficial for many collections that include Australian material.

Archival records have always been a powerful source of evidence for identifying the provenance of specimens within large natural history collections. Often the trail of archival breadcrumbs stop, leaving specimens disconnected from their object histories and contextual data. The practice of altering the corporeal state of specimens from wet ‘spirit’ preservation to dry study skins, or mounted specimens (45,46) further complicates efforts to connect individual

skins with the archival records. The research in this chapter, demonstrated that, by recognising specimens as human-made objects created through a craft practice that is repeated over time, elemental composition may connect ‘no-data’ specimens with their maker and the archival records of their field collection. This protocol has enormous potential worldwide for re-establishing the provenance of ‘no data’ specimens and enhancing the value of natural history collections.

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Conclusion

7.1 The problem

Zoological Museum collections are a great resource for research in numerous disciplines, each using specimens and their contextual data in various ways to investigate the living environment and human history within it.

Within this vast repository of biological and historic information some specimens lay underutilised. Their potential is constrained by the absence of contextual information that places the specimen, as a representative of its species, in geographic and temporal space. The recognition of that potential promotes the continued efforts to house and maintain them, but financial and storage pressures have encouraged museums to find ways to bring utility back to these 'no data' specimens.

The chemical composition of zoological specimens, with or without contextual data, impacts their utility in certain disciplines, e.g., biological and ecological research. The history of museum specimens as objects includes the application of preservative and pesticide chemicals. Specimen specific records of these historic applications are rare. Researchers must either exclude specimens from their research based on assumed object histories, or take a try-and-see approach in which analyses are undertaken on more specimens than required in the hope of gaining success. The first approach contributes to the underutilisation of museum specimens, the second leads to unnecessary destructive sampling and depletion of the biological archive.

7.2 The aims

The research documented in this dissertation, endeavoured to address both the absence of contextual data for historic specimens, and the lack of information about past chemical treatments for individual specimens. At the outset, the research aimed to achieve the following;

- develop an affordable and non-destructive analytical data collection protocol that can be readily applied in museum contexts;
- develop a data collection and analysis protocol that enabled comparison among historic museum specimens which can then be used to identify the treatment histories of groups of specimens;
- test the hypothesis that chemical residues remaining from the preservative and pesticide treatments that were applied after the animals capture from the wild, could be used to connect specimens with their maker and the contexts within which they were made;
- contribute to the history of zoological specimen collecting in Australia; and
- reclaim geographic and temporal contextual data for at least one 'no data' museum specimen.

7.3 The progress

Chapter 1 explored the problems associated with 'no data' specimens in museum collections and the value in re-establishing contextual data. This chapter discussed the success of past methods to re-establish lost contextual data and how the museum context affects the selection of those methods. Portable XRF spectroscopy was identified as suitable analytical tool for investigating the material evidence that is encapsulated within the specimen itself. This finding was the first step in achieving the aim of developing an analytical protocol that could be implemented in museums. Historic preservatives were presented as a resource for establishing provenance and several preservatives, detectable using XRF spectrscopy, were described.

In *Chapter 2*, a protocol was developed for collecting XRF data that recognised the chemical diversity of specimens while limiting deleterious physical impacts on them. Reference samples, representative of the historic preservatives described in Chapter 1, were created and analysed using inductively coupled plasma – mass spectrometry. The results were compared with the

portable XRF analysis of the same materials. Experimental parameters and data manipulation methods were established to avoid spectral artifacts and obtain robust comparative data on relative elemental content. This innovative protocol enabled reliable comparisons of analytical data collected from both preservative treated reference skins samples and from two historic skin specimens. Conclusive information about historic preservatives was revealed. To further mine the XRF data, these analyses required the use of principal component analysis (PCA).

Chapter 3 provided a critical evaluation of the nature of museum specimen data deficits. The loss of specimen data was presented according to three mechanisms: disassociation, atrophy and obscuration. These mechanisms focused on the intention behind actions that caused data loss, as a method to assessing the validity of surviving specimen data. This novel method of assessing data veracity may be helpful to researchers without a deep knowledge of museum operations and nineteenth century trade pathways.

Using this evaluation, the criteria for selecting historic specimens to be used in further research was identified, and historic specimens were chosen for the research outlined in the following chapters. The discussion within this chapter identified that study skins collected specifically for a collection or museum, and delivered with the minimal number of transfers of custody, were most likely to have the most reliable specimen data. *Thylogale* spp. specimens were selected representing the preservative practices of Kendall Broadbent (1837-1911), Robert Grant (c.1854-1923), Henry Sneddon Grant (c.1892-1944), and George Masters (1837-1912). The selection of specimens for analysis was the final step in formulating an experiment to test the use of the developed XRF protocol with PCA.

Chapter 4 unites the XRF/PCA protocol developed in Chapter 2 with selection criteria and museum specimens identified in Chapter 3. The results demonstrated that specimens collected by the same field collector clustered together based on similar variance from the mean in

elemental composition. Neither endogenous chemicals, location of field collection, nor different years of collection significantly impacted this finding. Through investigations of correlated elemental data, conclusions were drawn on which elements were applied at the same time, and hence, what chemical treatments were used.

This is the first study to demonstrate a connection between individual field collectors and the chemical composition of historical museum specimens. Results support the hypothesis that preservative residues can connect individual specimens with their maker. The findings established the potential of the protocol to identify specimens that were made by the same collector; and connect 'no data' specimens with others collected by the same field collector. The findings also demonstrated that the protocol was able to reveal the history of mixtures of preservatives used in the field in Australia. The outcomes of this chapter represented a significant advancement toward satisfying each of the research aims.

The future of the protocol is expected to benefit biologists and ecologists who seek to study numerous specimens collected at the same time and place. Humanities scholars will also profit from the re-establishment of lost museum specimen data.

Chapter 5 detailed the use of the established XRF/PCA protocol to determine the treatment history of dry specimen skins. The results showed that long-term storage in ethanol was characterised by high relative concentrations of Zn. This characteristic was observed to endure in mammal skins, and was identified in specimens that were reworked into dry skins over 100 years before the time of this investigation. This is the first time that such information has been derived using non-destructive analysis via portable XRF spectroscopy.

The research described in this chapter met the research aim of identifying of treatment histories for groups of specimens. Because preservative methods impact the success of genome studies, this is likely to be of interest as a non-destructive screening tool for research that utilises

museum specimens. These research outcomes have great potential for use in museums where knowledge of past treatments inform materials preservation treatments.

The final experiments, described in *Chapter 6*, explored the boundaries of knowledge that may be revealed by the XRF/PCA protocol. The potential of the protocol to connect field collectors with specimens, initially discussed in Chapter 4, was supported by the results described in this chapter. Specimens collected by the same field collector clustered together based on the variance in their elemental composition. Practices of George Masters were investigated to reveal that he used two discrete preservation techniques, one producing a 'wet' spirit specimen and the other a dry specimen. The final research aim of the research was then explored, to reclaim the field collection history of at least one 'no data' specimen. XRF data from bandicoot specimens collected by George Masters were compared with 'no data' specimens of two species, *Thylogale* spp. and *Perameles myosuros*. The results confirmed that the protocol was able to reconnect three 'no data' specimens with their collection histories and contextual data. This is the first time that elemental data, collected non-destructively, has been used to reattribute the provenance of zoological specimens.

7.4 The outcomes

This dissertation has met the research aims. It has;

- established a protocol that can reconnect 'no data' zoological specimens with the geographic and temporal information about their field collection by connecting three specimens with George Masters' field records;
- proven that the protocol can be used to reveal a treatment history of long-term ethanol storage in specimens that have been reworked into dry skins;

- contributed to knowledge of the field practices of George Masters, Robert Grant, and Kendall Broadbent. In doing so, this research has contributed to the history of zoological specimen collecting in Australia;
- proven the hypothesis that chemical analysis of preservative residues can be used to connect specimens to their makers; and
- provided a robust data collection and analysis protocol that can be used by natural history museum, and the people that use their collections, to support further research and the long-term preservation of specimen collections.

7.5 The limitations

This research was limited by the current capacity of handheld XRF instrumentation to detect low Z-weight elements in air. It is anticipated that future technological developments will offer the capacity to detect sodium (Na) and therefore the preservative, salt (NaCl).

It was noted that the differences between the practices of some field collectors were more complex to interpret than others. This is likely to limit the use of this protocol for identifying the preservative practices of every field collector.

7.6 The future

The results of chapter 6 suggest further research could be undertaken to understanding the role of Pb in zoological specimen preservation. The development of the protocol as a screening tool for materials conservation, and genomic studies is also worthy of future research.

Further investigation is warranted in broadening the present application of the protocol to reveal characteristics for identifying other preservation methods, e.g., formalin preservation. Such information would enhance the value of pXRF spectroscopy as a screening technique before further analysis.

Protocol functionality could be extended with the development of museum-wide spectral libraries of preservatives. This may be further enhanced with the use of more generalisable machine learning methods and PCA. The long-term storage of these analytical data files requires future research, to enable researchers to reference and develop these types of analytical methods using XRF and PCA.

The protocol applied to mammals in this research could be modified to study the elemental composition of bird specimens. This may contribute further to the history of zoological specimen collecting.

Future research to develop protocols using other non-destructive analytical techniques, such as near infrared spectroscopic, would provide complimentary compositional data and contribute to deeper understandings of preservatives applied to zoological specimen skins.

The data collection protocol applied in this research may be modified to enable non-destructive analysis in helium atmospheres. This would reveal the presence of sodium and may contribute to knowledge of the use of common salt (NaCl) in preservation of historic specimens.

Investigate the changes over time in the practice of a single field collector, or group of collectors. This may be particularity interesting if the time period chosen encapsulates the impact of new technologies or new cultural influences.