

ARE YOU UP TO THE TUSK? IDENTIFYING IVORY SPECIES USING RAMAN SPECTROSCOPY AND MASS SPECTROMETRY

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Introduction

Analytical chemistry research is needed to improve techniques for identifying ivory, a protruding tooth, by origin and species while minimizing sample size. Poaching of African elephants (*Loxodonta africana* and *Loxodonta cyclotis*) and Asian elephants (*Elephas maximus*) is an issue that ultimately threatens their survival; this makes identifying the origin of elephant ivory even more important not only for addressing poaching and ivory trading, but also for assisting museums overseeing ivory artifacts.¹

To combat elephant poaching, enforcement of African elephant ivory trade regulations under CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) has intensified.¹ These regulations greatly impact the movement of museum exhibitions, requiring staff to identify the ivory's originating species.¹ While species identification is important for legal documentation, determining the origin of ivory artifacts is crucial for historical, curatorial, and art conservation purposes to achieve a better understanding of its unique context. Culturally significant ivory artifacts pose an additional challenge, as it is imperative to use minimally invasive sampling techniques.

Bone and antler have a similar composition to ivory, and were included in this study as model systems due to greater availability.² This study uses Matrix Assisted Laser Desorption/Ionization - Time of Flight (MALDI-TOF) mass spectrometry, Raman spectroscopy, and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) to differentiate between various species using bone, antler, and ivory.

Sample Preparation

1. Optimize sampling using a Dremel stylus to create powder and cross section samples.
2. Prepare samples accordingly for each of the three techniques.
 - Raman Spectroscopy
 - MALDI
 - ICP-MS
3. Conduct analysis using bone and antler samples to optimize parameters.
4. Apply optimized procedures to ivory samples.



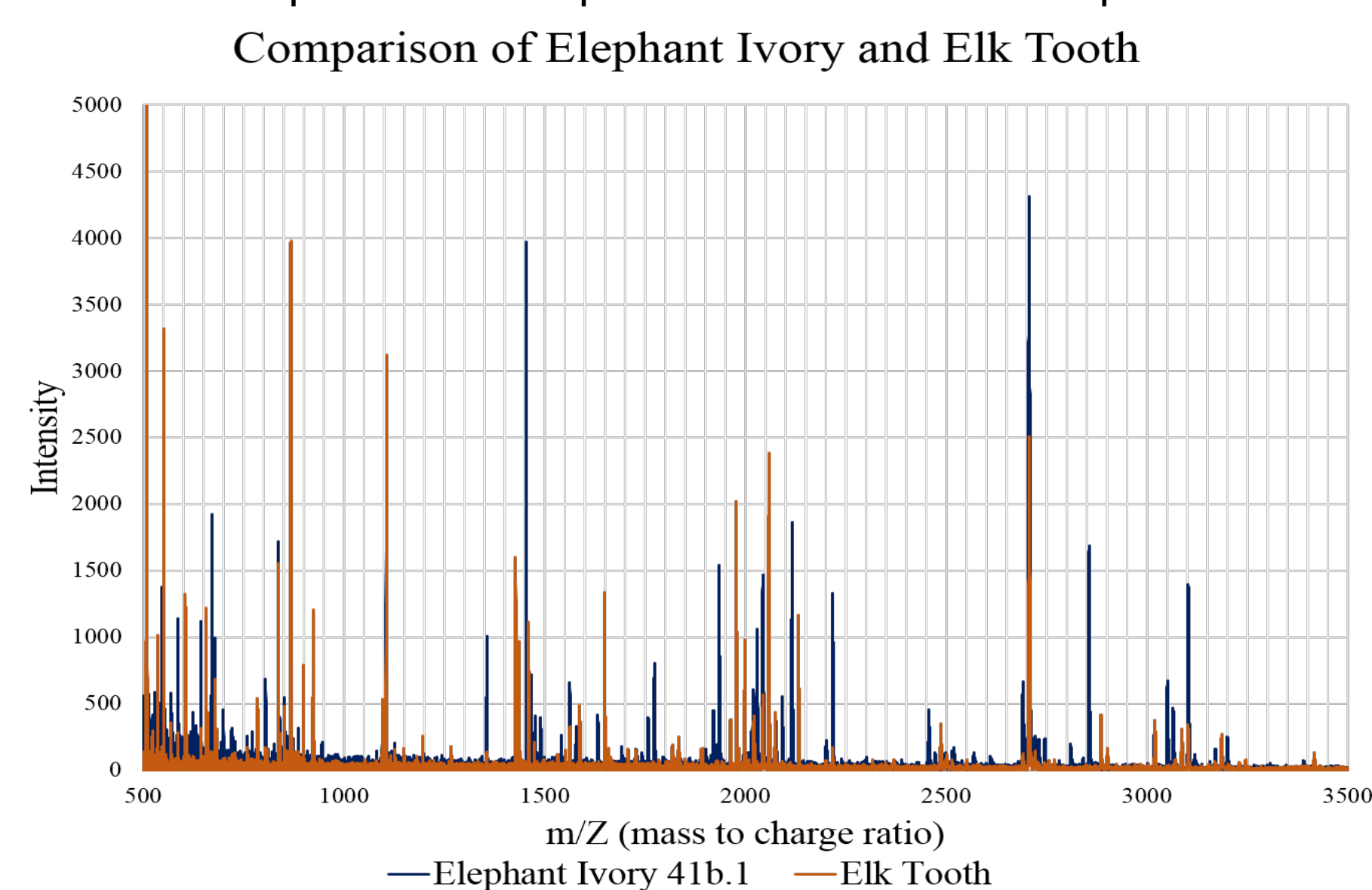
Minoan Snake Goddess, gold and likely elephant ivory
16th century BCE (Late Minoan)
The Walters Art Museum, Baltimore
71.1090



"Pokal" with Royal Portraits, mammoth ivory
2nd half of 18th century (Russian)
The Walters Art Museum, Baltimore
71.477

MALDI-TOF

MALDI-TOF mass spectrometry targets large molecules such as peptides. MALDI enables us to measure the masses of the peptides produced by breaking down the collagen protein found in ivory using trypsin, a common protease. These masses are used to identify specific markers found in various mammals.⁹ Though this is a destructive technique, it requires minimal sample size compared to other techniques.

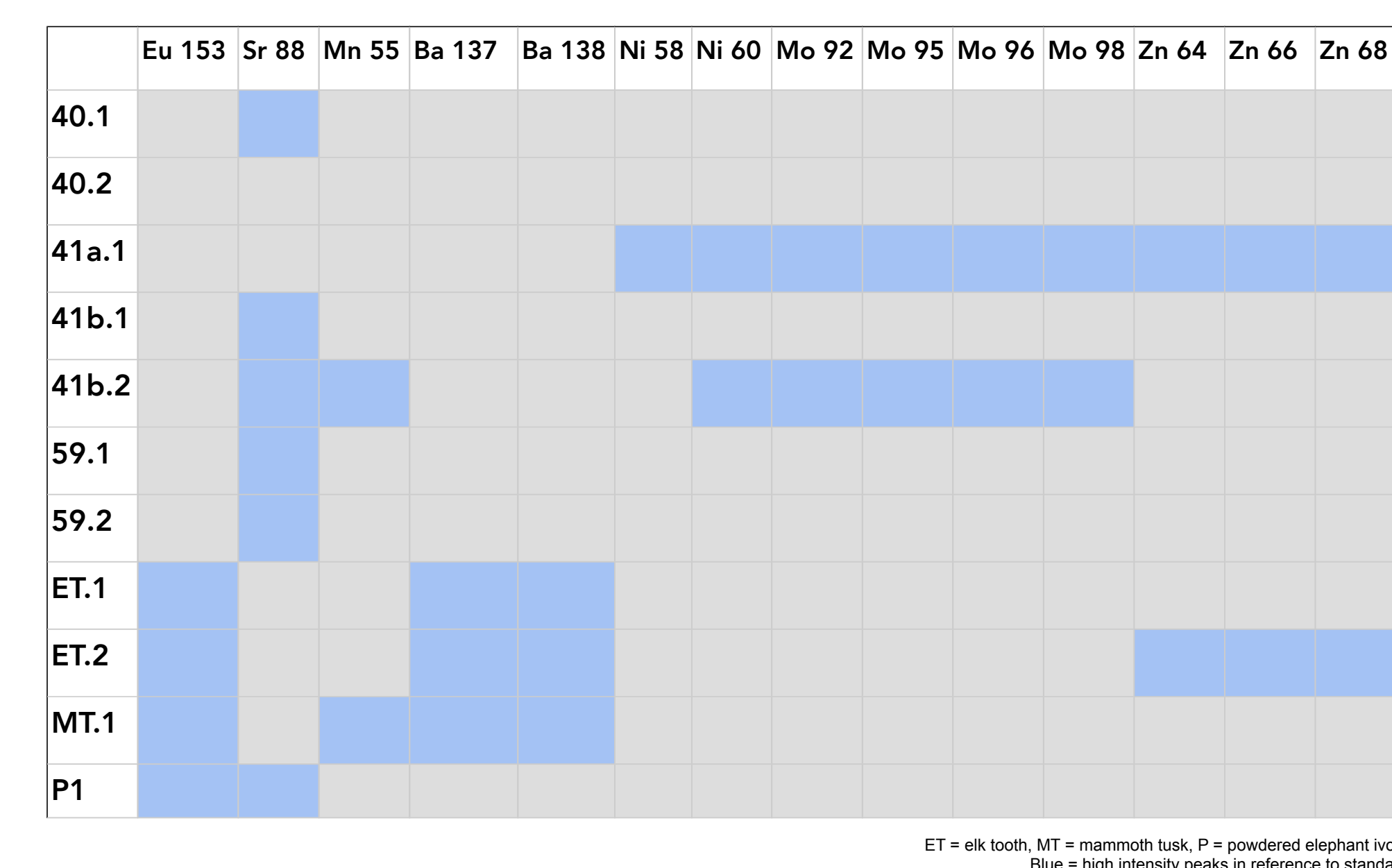


Sample	Cet1	(A)	(B)	(C)	Cet2	(D)	(F)	(G)
40.1	1105.7	nd	nd	1580.2	nd	nd	2854.4	nd
40.2	1105.7	1251.9	1453.9	1580.1	1541	2116.4	2853.8	3015.9
41a.1	1105.9	nd	1454.1	1580.2	1541.2	2116.7	2853.9	3015
41b.1 #1	1105.7	1251	1453.9	1579	1540	2116.4	2853	3015
41b.1 #2	1105.9	nd	1454.2	nd	1541.2	2115.8	2854.8	nd
41b.2	1105.7	nd	1453.9	1579	1540	2116.4	2853	3015
59.1	1105.9	nd	1454.2	1580.2	1541.2	2116.7	2854.5	nd
59.2	1105.9	nd	1454.2	1579	1541.2	2116.7	2854.5	nd
ET.1	1105.8	nd	1427.9	nd	n/a	2132.4	2885.6	3033
ET.2	1106	1197.1	1428.3	1551.4	n/a	2131.9	2884.4	nd
MT.1	nd	nd	nd	nd	nd	nd	nd	nd
P.1	1105.9	nd	1454.2	1580.3	1541.2	2116.8	nd	nd
P.2	1106.1	nd	1454.4	nd	nd	nd	nd	nd

nd = non-detectable, n/a = non-applicable

ICP-MS

ICP-MS is used to identify trace elements such as transition metals that are present within an aqueous sample. Applied to ivory, this reflects the various minerals present in the species' environments.⁸



Conclusion

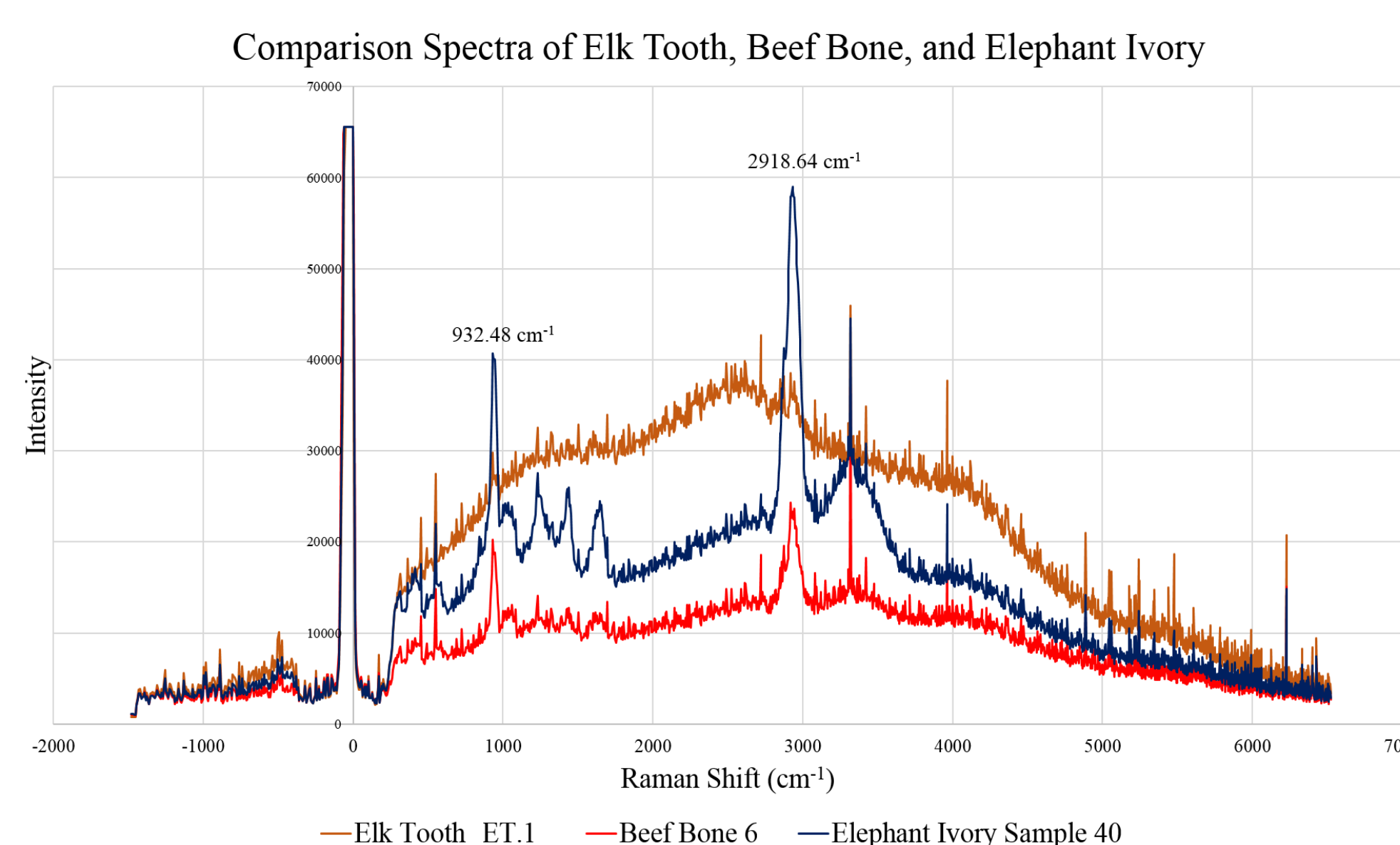
Within elemental analysis of ICP based on trends noticed in African and Asian elephants, there were some notable differences between the elephant ivory samples. In order to verify significant differences in the concentrations of particular elements, further research would need to be performed with other ivory samples. The same elements were analyzed within elk tooth samples and it should be noted that the elements are not specific to North America.

From the analysis using MALDI it can be concluded that mammalian species can be differentiated through ivory samples. Through the markers compiled by Dr. Daniel Kirby, it was confirmed that the samples analyzed were elephant and elk. Due to the mammoth sample being fossilized, the low proportion of remaining collagen made the spectra inconclusive. This confirms that peptide analysis techniques are not suitable for fossilized samples. There were also inconclusive differences noted between African and Asian elephants. Further analysis of spectra and perhaps looking at different markers would possibly prove to be useful.

Through the use of Raman spectroscopy, it could be verified that the hydroxyapatite $\nu(\text{PO})$ and collagen $\nu(\text{CH})$ peaks could be seen throughout the elephant ivory samples. These same peaks are not strongly apparent in bone, fossilized mammoth ivory, or elk tooth samples. Most of the data taken showed a low signal-to-noise ratio due to the fluorescence of each sample. Completely photobleaching the samples prior to spectral collection would resolve this error in future studies.

Raman

This form of spectroscopy allows one to characterize both organic and inorganic components within a sample by detecting changes in polarizability. Previous literature has shown the ability to characterize ivory by comparing peak intensity ratios between the organic $\nu(\text{CH})$ peaks in collagen and inorganic $\nu(\text{PO})$ peaks in hydroxyapatite.⁶



	$\nu(\text{PO})$ cm^{-1}	$I(\text{PO})$	$\nu(\text{CH})$ cm^{-1}	$I(\text{CH})$	Ratio $I(\text{CH}) / I(\text{PO})$
Elephant Ivory 40	932.48	40674.11	2931.13	58957.17	1.469
Elephant Ivory 41a	932.48	27826.76	2918.64	41132.10	1.585
Elephant Ivory 41b	932.48	27237.02	2918.64	42498.24	1.653
Elephant Ivory 59	932.48	22266.91	2918.64	28361.06	1.309
Beef Bone 4	932.48	14259.06	2918.64	18285.85	1.087
Elk Tooth ET.1	932.48	18186.04	2918.64	23079.17	1.251

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