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3th International Conference on Forensic Research and Technology
Octuber 6-8, 2014
San Antonio
USA

Chemical Element Levels as a Methodological Tool in Forensic Science

GIANNI GALLELLO

(co-authors: Julia Kuligowski, Agustín Pastor, Agustín Diez, Joan Bernabeu)

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INTRODUCTION

Alterations on the structure and chemical composition of human bones and tissues can be generated by:

- Human activities
- Accidents
- Cremations
- Funerary rituals

<u>**General aim**</u>

We are looking for trace elements and rare earth elements (REE) potential characteristics to propose novel methodological approach applicable to forensic science scenarios.

<u>**Specific aims**</u>

I. Define a strategy to select bone samples correctly .

II. Heavy metal results (e.g. arsenic -As-, mercury- Hg- and lead-Pb-) correct interpretation for forensic science approaches.

I.MATERIAL AND METHOD

I.1 ANALYTICAL TECHNIQUES

To determine trace elements and rare earth elements (REEs), techniques based on atomic spectrometry are employed.

- *Inductively Coupled Plasma Optical Emission Spectrometry* (ICP-OES)

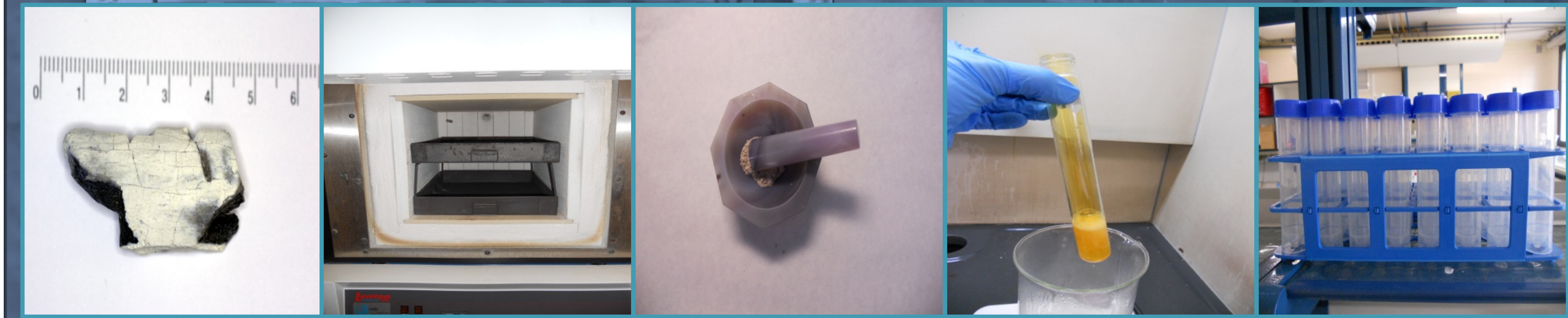
- *Inductively Coupled Plasma Mass Spectrometry* (ICP-MS)

- *Cold-Vapor Atomic Absorption Spectrometry “mercury direct measurement” (CV-AAS)*

- *Hydride Generation Atomic Fluorescence Spectrometry* (HG-AFS)

I.2 Analytical Methodology

Sample Preparation



Analyzed samples during the last two years

TYPES	NUM.
BONES	226
BONE SURFACE	141
SOILS	136
WOODS	10
HUMAN TISSUES	7
TEXTILES	2
TOTAL	522

I.3 Statistics

Multivariate Statistics

- Cluster Analysis (CA)
- Principal Component Analysis (PCA)
- Partial Least Squares Discriminant Analysis (PLS-DA)

Hypothesis Testing

- Standard Deviations
- Dixon Test
- Average Comparison
- Propagation of Error

II. METHODOLOGY and QUALITY

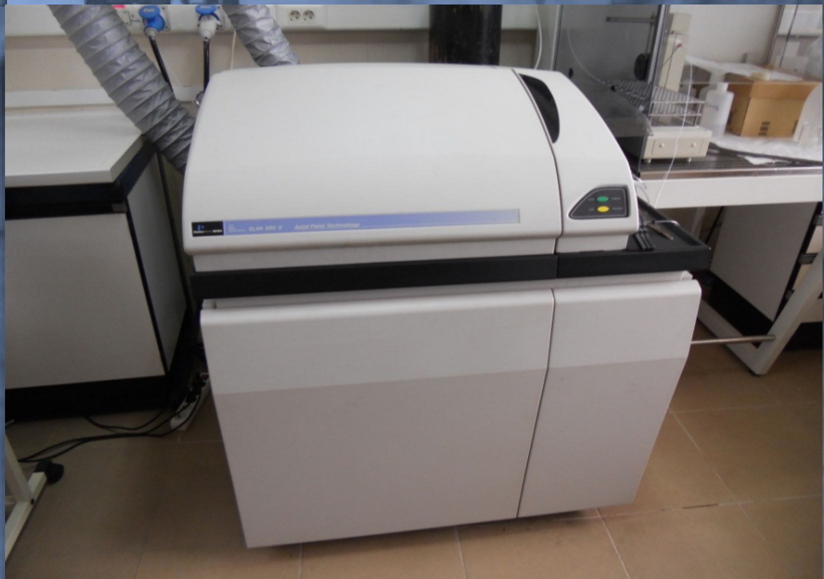
II.1Measurement and Elemental Parameters (ICP-OES)

Argon Flows	• **Plasma 15 L/min** • **Auxiliar 0.2 L/min** • **Nebulizador 0.80 L/min**
Power	•**1300 Vatios**
Plasma position	• **Radial (Ca, Sr, Mg)** • **Axial (Elementos traza y REEs)**

ELEMENTS	W.L. [nm]	LOD (µg/g)	LOQ (µg/g)	R^2
Ca	317.933	1600	5400	0.9996
Sr	421.552	4	13	0.9995
Mg	285.213	0.4	1.3	0.9999
Zn	206.2	0.4	1.2	0.9998
Cu	327.393	0.11	0.4	0.9999
Ba	233.527	0.08	0.3	0.9999
V	290.88	0.07	0.2	0.9999
Mn	257.61	0.14	0.5	0.9997
Cd	228.802	0.05	0.17	0.9998
Pb	220.353	0.6	1.8	0.9996
Cr	267.716	0.06	0.2	0,9999
Co	238.892	0.01	0.3	0,9999
Ni	231.604	0.17	0.6	0,9998
La	408.672	0.04	0.13	0.9999
Ce	413.764	0.03	0.11	0.9995
Pr	390.844	0.4	1.2	0.9995
Nd	406.109	0.2	0.8	0.9998
Sm	359.26	0.2	0.7	0.9994
Eu	382.967	0.003	0.01	0.9996
Gd	342.247	0.004	0.014	0.9995
Tb	350.917	0.03	0.08	0.9997
Dy	353.17	0.02	0.07	0.9995
Ho	345.6	0.008	0.03	0.9997
Er	337.271	0.11	0.4	0.9995
Tm	346.22	0.04	0.12	0.9997
Yb	328.937	0.003	0.009	0.9999
Lu	261.542	0.003	0.009	0.9996
Sc	361.383	0.002	0.006	0.9999
Y	371.029	0.006	0.02	0.9999
Ru*	240.272	-	-	-
Be*	313.107	-	-	-

II.2Measurement and Elemental Parameters (ICP-MS)

Presión de vacio	5x 10^{-5} torr
Argon Flow	0.92 L/min
Power	1100 Watts
Plasma setting	15 sec

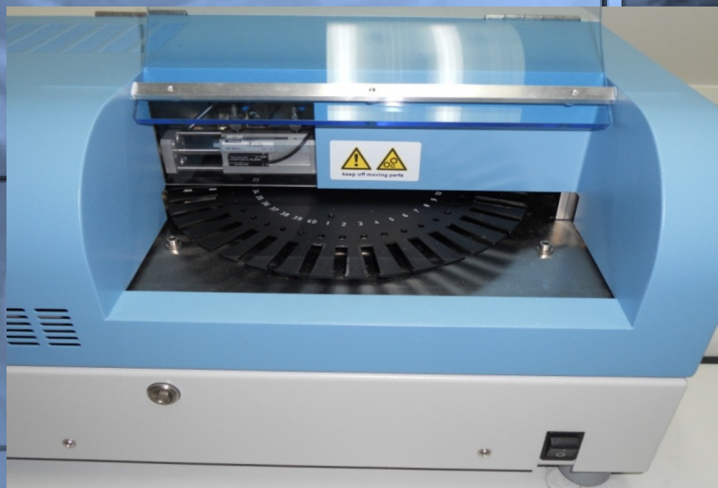


ELEMENTO	SIMBOLO	ms [Da]	LOD (µg/g)	LOQ (µg/g)	R^2
Lantano	La	139	0.0004	0.0014	0.9997
Cerio	Ce	140	0.0005	0.0018	0.9997
Praseodimio	Pr	141	0.00010	0.00032	0.9997
Neodimio	Nd	142	0.0003	0.001	0.9985
Samario	Sm	152	0.00035	0.0011	0.9999
Europio	Eu	151	0.00005	0.00018	0.9998
Gadolinio	Gd	158	0.00015	0.0005	0.9998
Terbio	Tb	159	0.00005	0.00017	0.9977
Disprosio	Dy	162	0.00001	0.00004	0.9998
Holmio	Ho	165	0.00003	0.00011	0.9983
Erbio	Er	166	0.00013	0.0005	0.9999
Tulio	Tm	169	0.000016	0.00005	0.9985
Iterbio	Yb	172	0.00007	0.0002	0.9999
Lutecio	Lu	175	0.000017	0.00006	0.9991
Escandio	Sc	45	0.013	0.04	0.9998
Itrio	Y	89	0.0005	0.0016	0.9996
Bario	Ba	138	0.00019	0.0006	0.9992
Bismuto	Bi	209	0.0006	0.002	0.9999
Cadmio	Cd	111	0.00017	0.0006	0.9995
Cromo	Cr	52	0.01	0.3	0.9986
Cobalto	Co	59	0.0004	0.0014	0.9986
Cobre	Cu	63	0.009	0.03	0.9991
Plomo	Pb	207	0.0007	0.002	0.9996
Litio	Li	7	0.0002	0.0008	0.9994
Manganeso	Mn	55	0.004	0.015	0.9983
Molibdeno	Mo	95	0.0011	0.004	0.9998
Niquel	Ni	60	0.007	0.02	0.9996
Estroncio	Sr	88	0.0005	0.0015	0.9999
Talio	Tl	205	0.00008	0.0003	0.9999
Titanio	Ti	47	0.09	0.3	0.9999
Vanadio	V	51	0.7	2	0.9985
Zinc	Zn	64	0.015	0.05	0.9998
Rodio	Rh*	103	-	-	-

II.3Measurement and Elemental Parameters (AAS-CV)

N.	Time	Temperature
1	00:01:00	200°C
2	00:02:00	650°C
3	00:01:00	650°C
Starting Maximum Temperature 200°C		
Purge 50 seconds		

ELEMENT	W.L.	LOD(µg/g)	LOQ (µg/g)	R^2
Hg	253.65	0.0006	0.002	0.9999



II.4 Measurement and Elemental Parameters (HG-AFS)

Argon Flow	250 mL/min
$NaBH_4$ Flow	4.5 mL/min
Fist Discharge mA	27.5
Second Discharge mA	30
Element	As

ELEMENT	W.L.	LOD (µg/g)	LOQ (µg/g)	R^2
As	200nm	0.02	0.08	0.9999

II.5 Certificate and Control Samples

To carry out a proper control on the quality, reliability and validation of the results, we have continuously employed CRMs and control samples.

NIST - 1400 Bone Ash (ICP-OES, ICP-MS and HG-AFS)

NIM- GBW07408 Soil (ICP-OES and ICP-MS)

NIST -1633C Coal Fly Ash (Mercury AAS-CV)

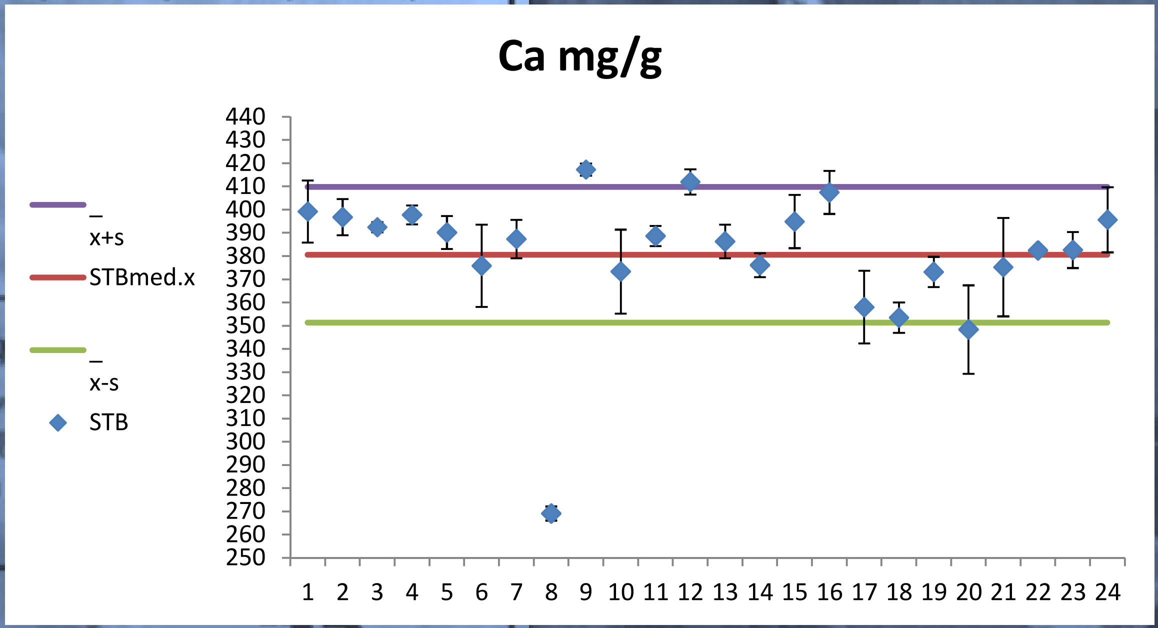
Control Samples “MF” (ICP-OES y ICP-MS)

II.6 Reliability and precision of the analytical methodology employed

BONE ASH NIST 1400 values expressed in µg/g. Obtained Values (OBT. V.). Certificate Values(CERT.V.). Standard Deviation (*s*). * mg/g values.

El.	OBT. V.	*s*	V. CERT.	*s*
Ca*	380	30	381.8	1.3
Mg	7190	320	6840	130
Sr	280	30	249	7
Zn	178	21	181	3

BONE ASH NIST 1400 obtained values during each analysis



III.BONES AND DIAGENESIS

Post-mortem alterations (weathering, dissolution, precipitation, microbial attack, mineral replacement, ionic substitution, recrystallization and isotopic exchange) in bones are commonly referred to as diagenesis.

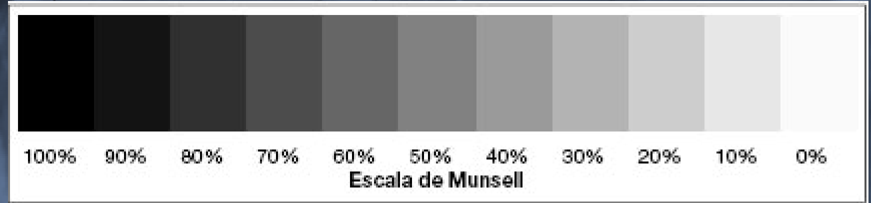
In many cases a previous impact on the structure and chemical composition of the bones is induced by cremation during funerary rituals, cooking habits or other human activities.

As a development of those prior methodological works, more recently, rare earth elements (REEs) analyses have been in general performed to monitoring the impact of diagenetic processes in bones.

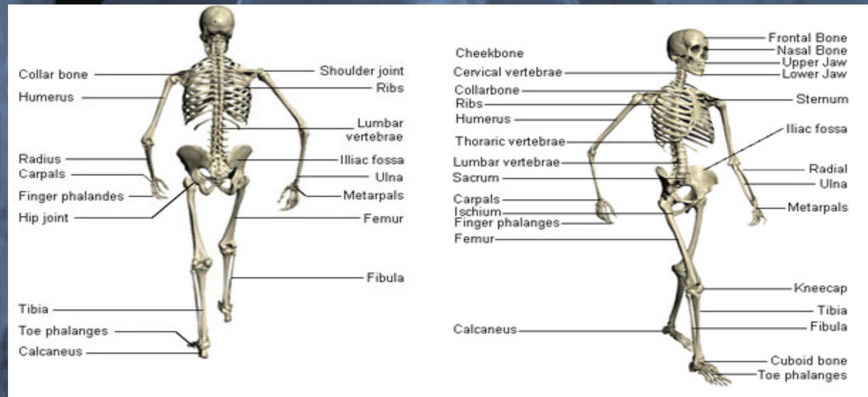
The limits of chemical analysis methodological approaches are linked to the control of the variables intervening during the post-depositional processes that can mislead data interpretations.

IV.BURNED BONES METHOD

A sampling strategy to control archaeological bone contamination due to diagenetic and taphonomic processes has been designed.



Cremated bones that were suffered different thermal impact were sampled. They belong to different part of a “thermal” gradient.

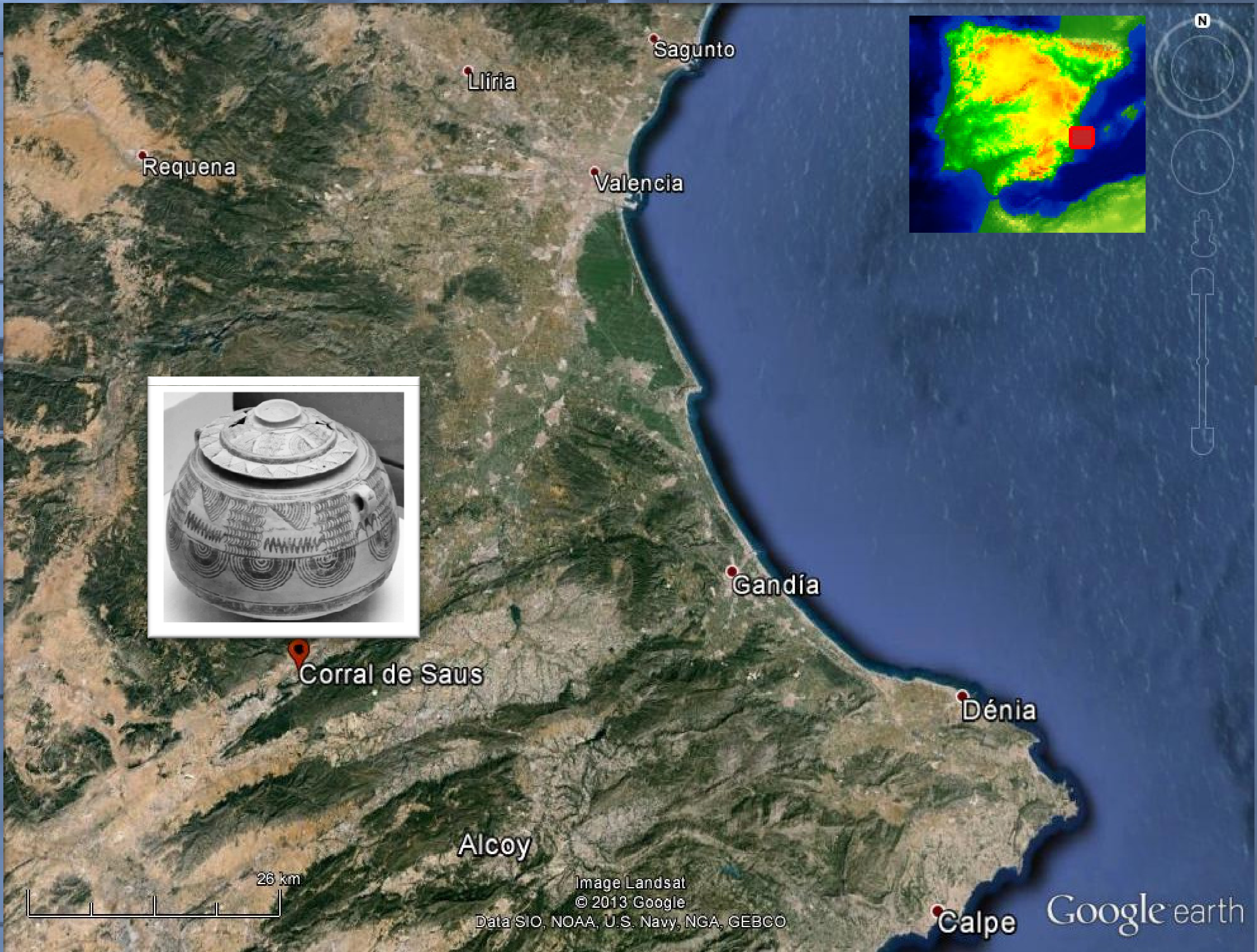


Different skeletal sectors for each individual have been sampled.

•Iron age Necrópolis of Corral de Saus. Cremated bones.

IV.1 Results

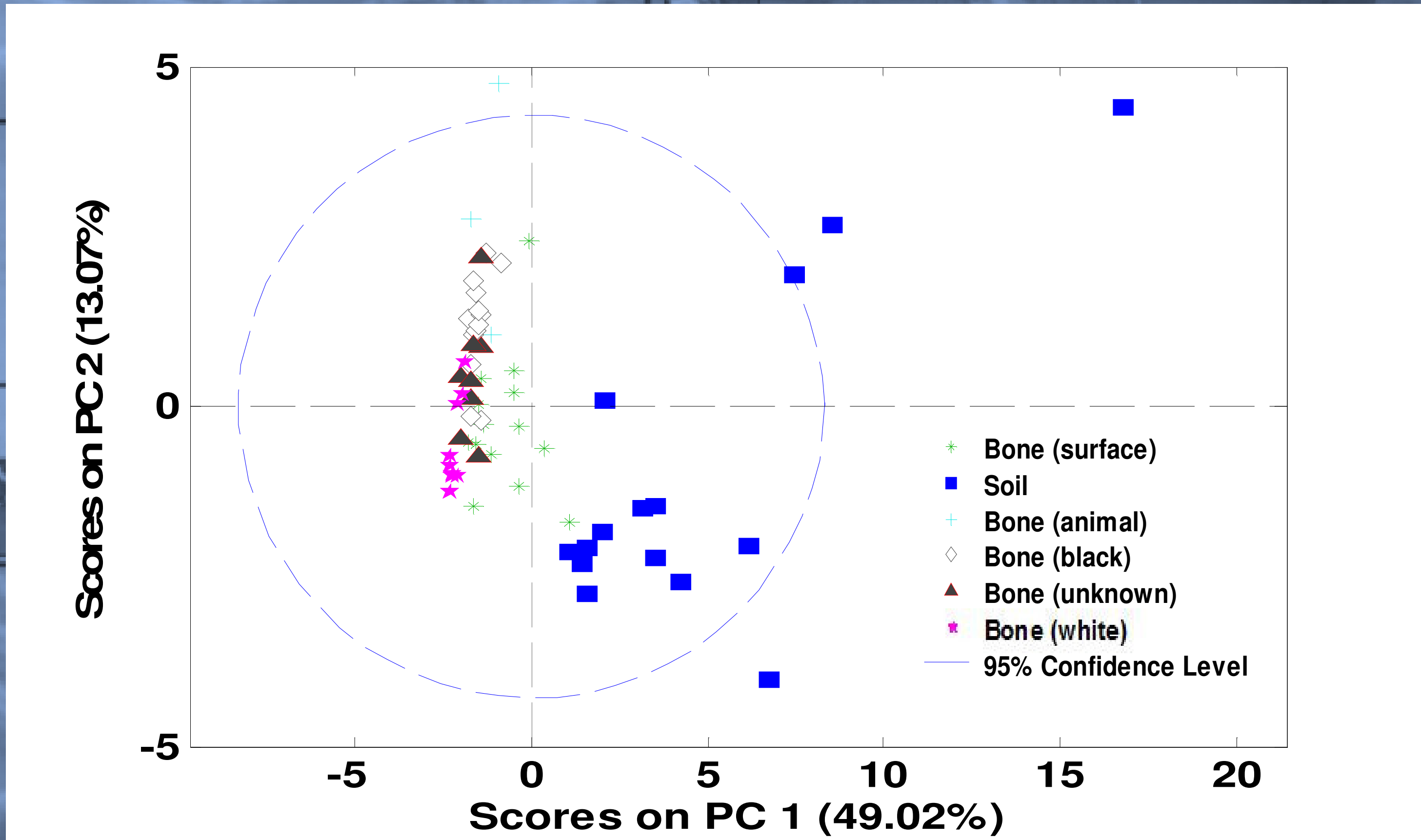
Corral de Saus



MUESTRA	UE	N. S.I.P	SEXO*	METAL*	CERAMICA IMP.*
21; 85	24280	61464	M	Hierro y Bronze	B.n.s III
22; 23; 24; 25; 26	24291	61429	M	Hierro	
37; 38	24277	62627	M		
39; 40; 41	24282	61381	ADULTO	Hierro	
44; 45; 46	24292	61430	M	Hierro	
18; 19; 47; 48; 49; 50	24288	61414	M	Hierro	
54; 55; 56; 57; 58; 59	24274	61399 "Tumba de la Sirena"	M	Hierro y Bronze	Áticas/b.n.s III y campan.
60; 61; 62; 63; 64; 65; 66	24285	CUA A16	ADULTO		
67; 68	24273	61377	F		
69; 70; 71; 72; 73; 75	24283	61396 "Tumba de la Damita"	M	Hierro	Áticas y campan.
80; 81; 82	24286	61413- Go12	ADULTO	Hierro	
84; 83A; 83B	24281	61389	M		
31; 32; 34; 35; 36	24275	61461	M	Hierro	
27; 28; 29; 30	24289	61386	F		
42; 43	24279	61401	M	Hierro y Plomo	B.n.s III
76; 77; 78	24276	61462	M	Hierro	
51; 52; 53	24295	61420	M		

- 17 individuals dated III-II centuries BC have been analyzed.
- The obtained dataset contained 65 samples and 22 variables (elements).

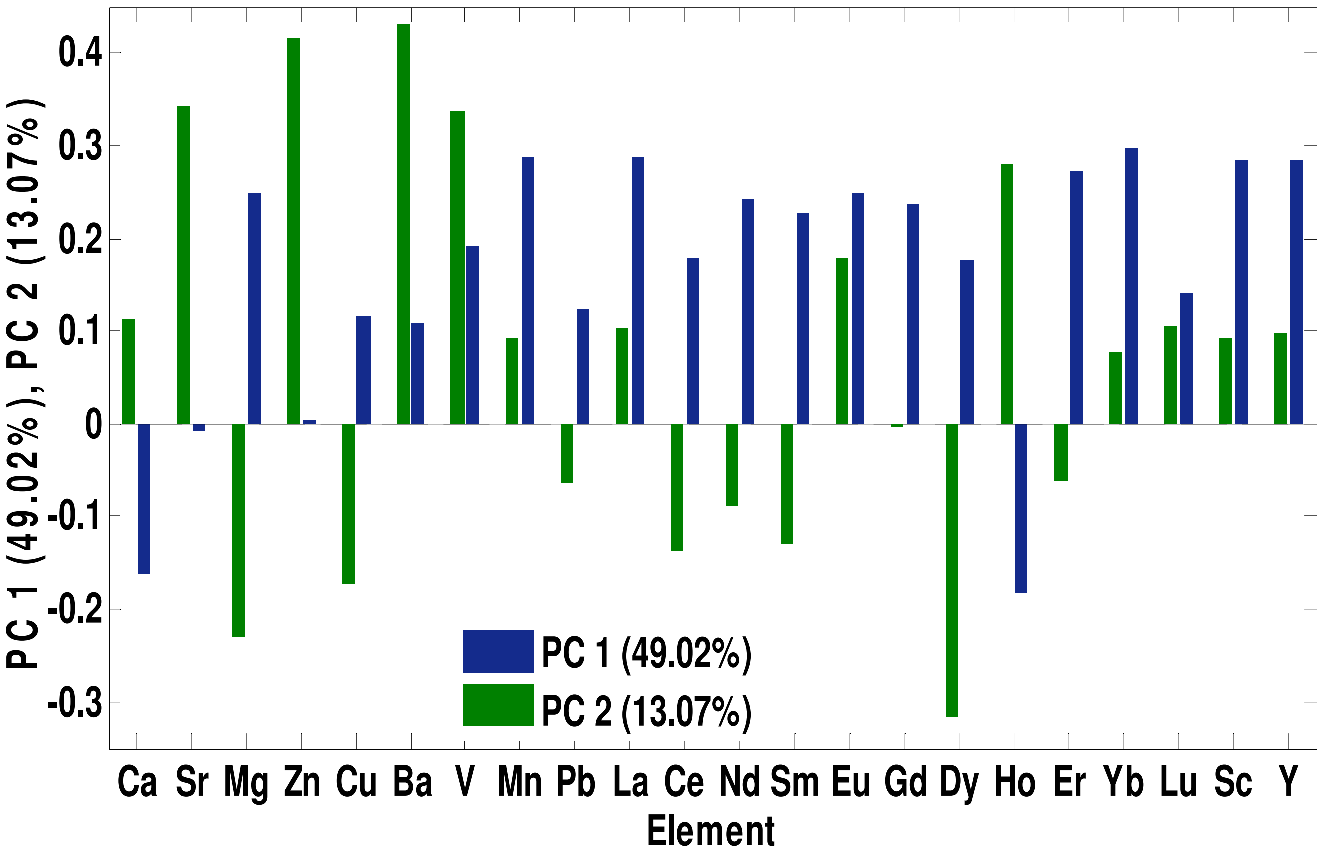
Revealing the impact of diagenetic factors applying PCA



•Bone samples from the outer bone layer are located between soil samples and bone samples in the direction of PC1.

•PC2 captures variance explaining differences between white and black bone samples (all from the inner bone part).

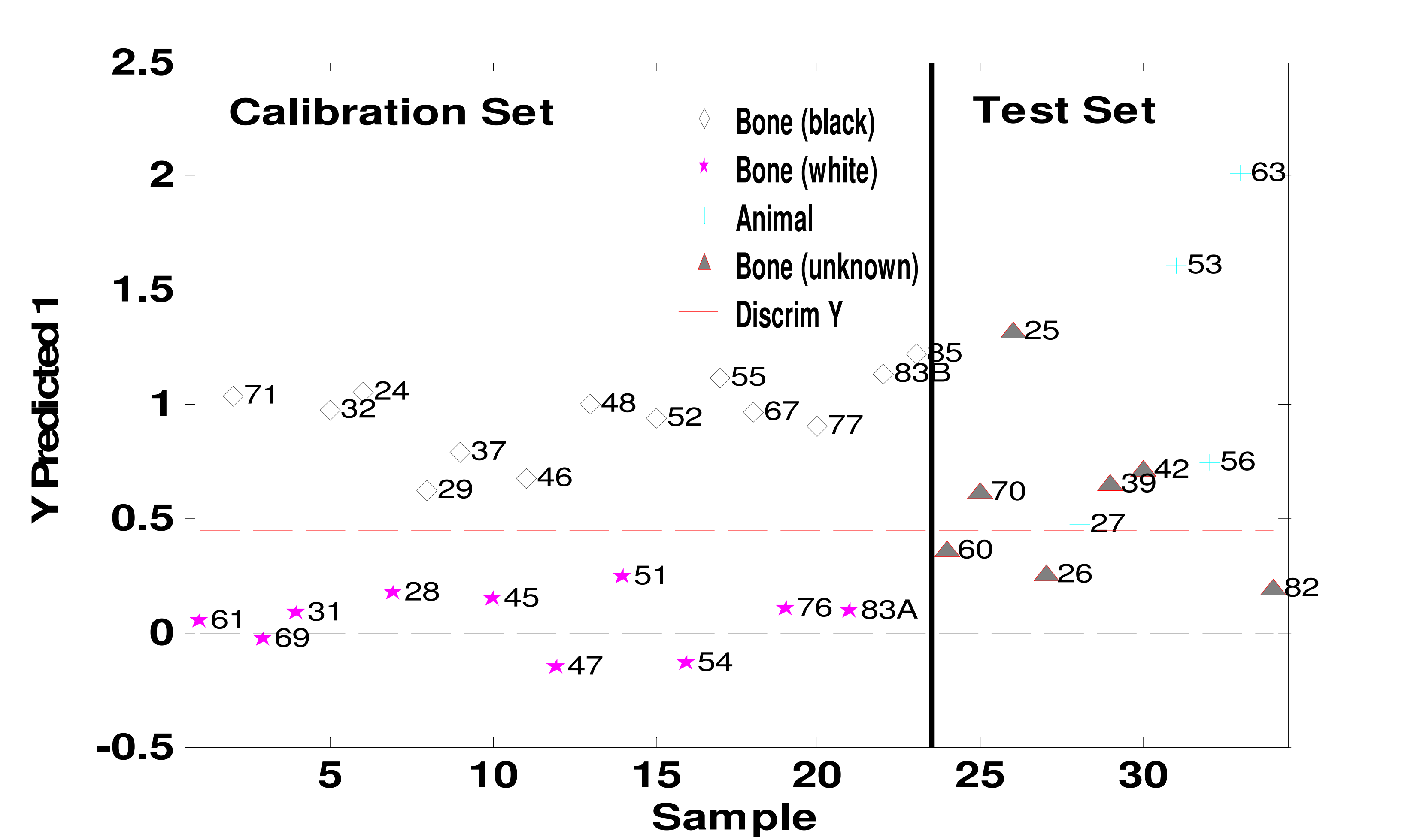
Loadings. Contribution of each variable (i.e. elements) to the calculation of PC1 and PC2 are represented.



•Ca show higher concentrations in bone samples than soil samples.

•Concentrations of Mn, La, Er, Yb, Sc and Y are lower in bone samples and higher in soil samples.

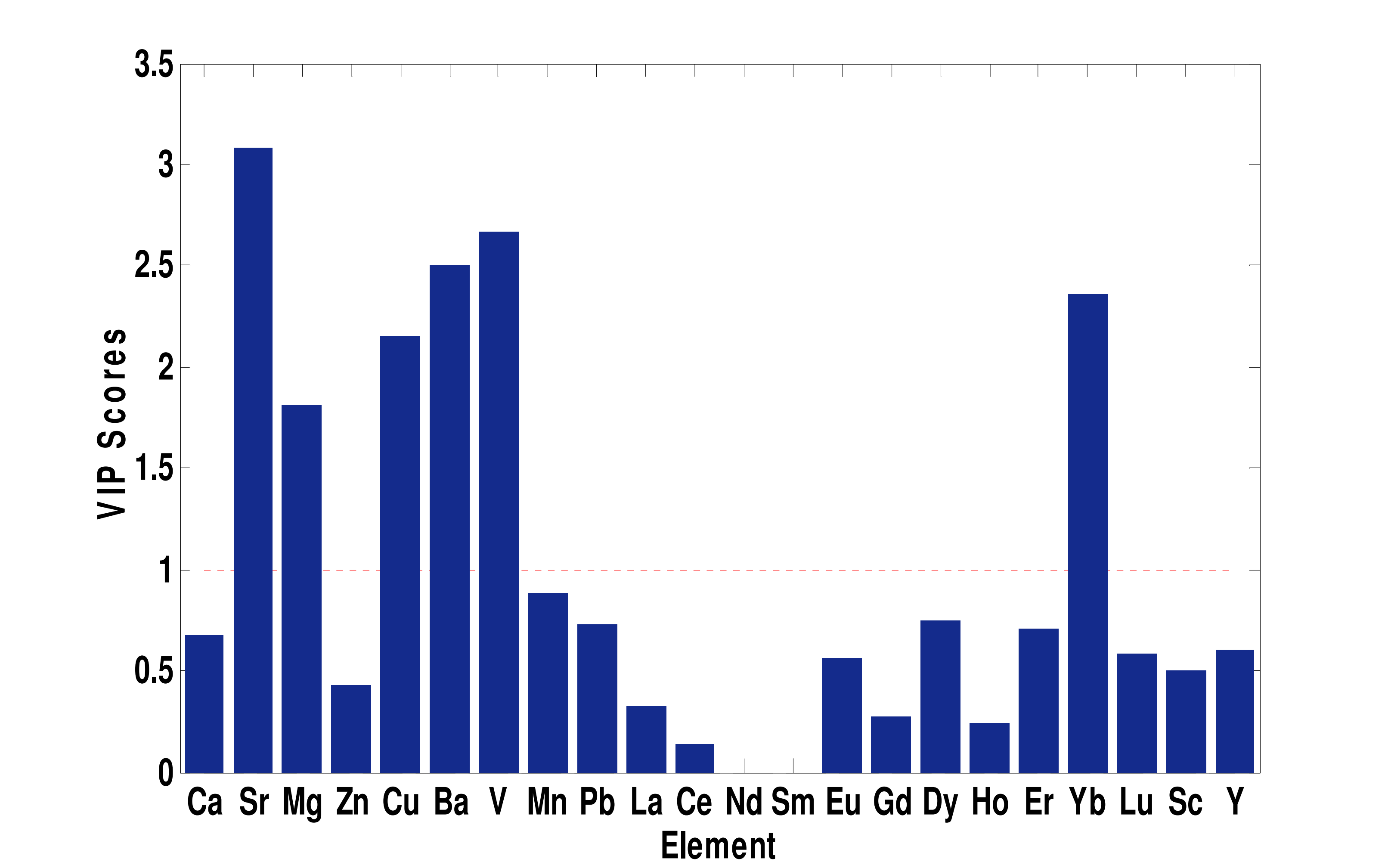
Corral de Saus Cremated bones classification (PLS-DA).



•Good class separation between black and white bones was obtained using a PLS-DA model .

•The model was applied to a set of bone of unidentifiable burning and some animal bone in dependence on their chemical characteristics.

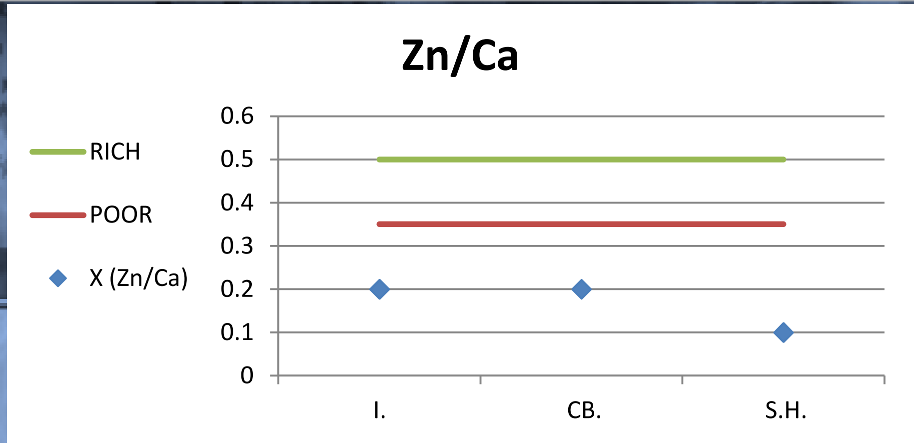
Variable Importance in Projection (VIP) scores for the calculated model .

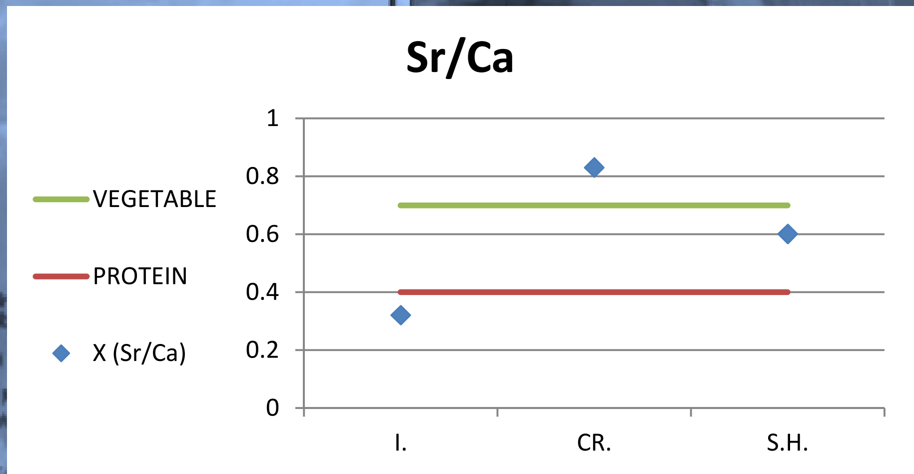


- Sr, Mg, Cu, Ba, V and Yb were the elements with the highest impact on the PLS-DA model.
- Concentrations are higher in carbonized than incinerated bones.

IV.2 Population Lifestyle (diet) reconstruction, differences depending on the bone class: an example

El/Ca Corral de Saus samples. Zn/Ca (rich and poor protein intake), Sr/Ca (vegetable and animal protein based diet). Incinerated bones (I.), carbonized bones (CB.), bone surface (S.H.).





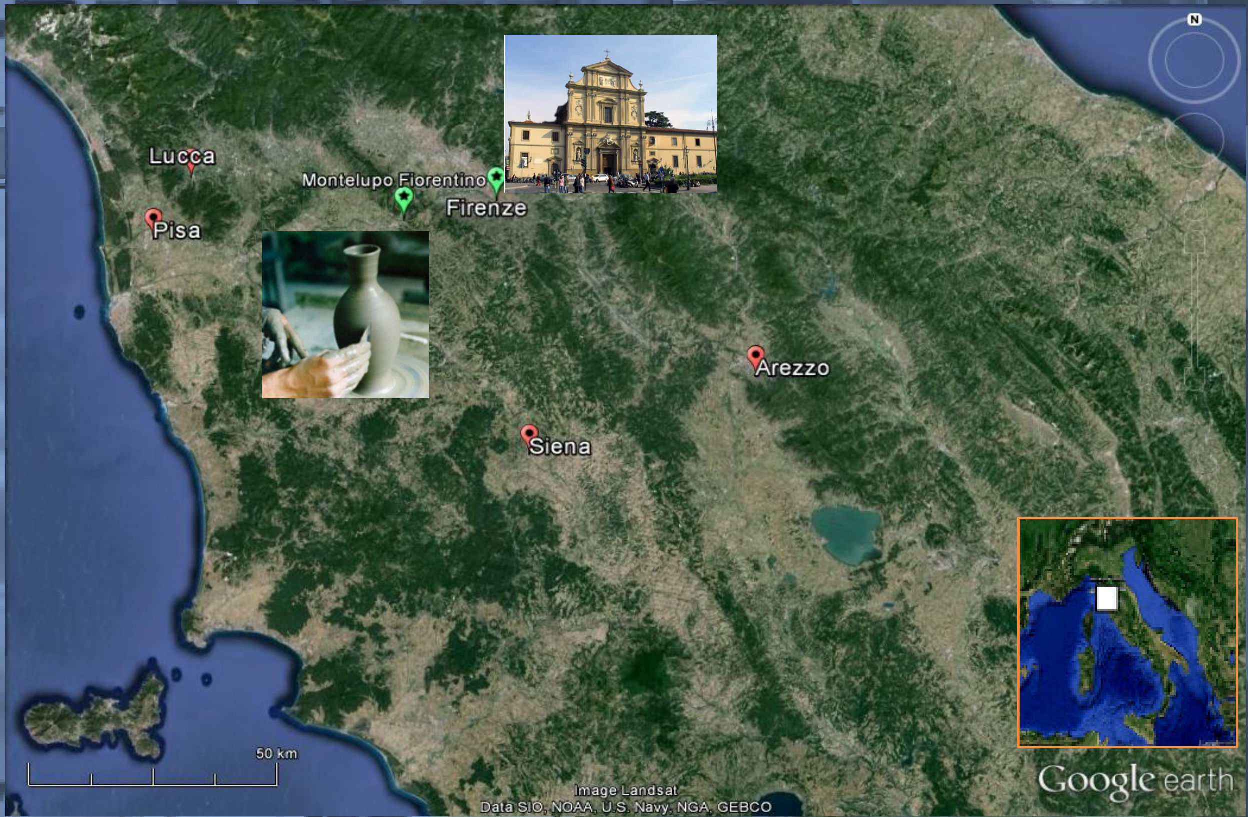
V. FORENSIC ARCHAEOLOGY CASES

- Some forensic archaeological studies have focused their efforts on the determination of heavy metals in human remains in order to establish intentional or unintentional poisoning as the cause of death of some individuals.
- Arsenic (As), Mercury (Hg) and Lead (Pb) have been the more attractive elements from scholars investigating the cases of poisoning in ancient populations or in famous people of antiquity.

Renaissance Humanists and Medieval Ceramist

We have selected two cases:

- The first case about the Italian Renaissance Florentine humanists, Giovanni Pico della Mirandola, Angelo Poliziano and Girolamo Benivieni.
- The second case from the medieval excavation of Montelupo Fiorentino town (Florence, Italy), famous for its ceramic production (an individual that archaeologist believe to be a ceramist).



Humanist Florentine individuals chemical analysis of toxic metals (As, Hg) have been carried out to clarify if their death was due to poisoning, and to find out the biological relevance of elemental concentrations not conditioned by *post-mortem* processes .

Pico della Mirandola
(1463-1494)

Benivieni
(1453-1542)

Poliziano
(1454-1494)

About ceramist the aim have been understand, through metal analyses (As, Hg, Pb), if its high concentrations levels are due to the long exposure to toxic agents (colors) or if the results are conditioned by *post-mortem* processes.

As y Hg (µg/g) values in Giovanni Pico Della Mirandola, Girolamo Benivieni y Angelo Poliziano.

SAMPLE	DESCRPTION	As	±	σ	Hg	±	σ
PICO							
5	RIB	1.50	±	0.95	0.53	±	0.03
16	SKIN LEFT FOOT	LOQ	±	-	0.52		0.05
22	RIB	LOQ	±	-	0.02	±	0.01
BENIVIENI							
8	PHALANX	0.34	±	0.06	0.09	±	0.03
11	SOFT TISSUES	0.23	±	0.49	1.04	±	0.27
12	RIB	LOQ	±	-	0.12	±	0.14
14	SKIN ANTERIOR RIGHT FEMUR	0.59	±	0.12	0.12	±	0.04
17	CLOTH PELVIS AREA	0.76	±	0.24	0.94	±	0.48
18	SKIN RIGHT HAND CARPATI	LOQ	±	-	0.39	±	0.10
19	LEFT TEMPLE BIB	3.97	±	0.48	4.25	±	0.78
20	LEFT TEMPLE	2.45	±	1.60	1.93	±	1.03
23	SOFT TISSUES RIGHT HEEL	0.99	±	0.36	0.76	±	0.45
24	SOFT RIGHT MUSCLE TISSUE	0.75	±	0.06	0.65	±	0.44
POLIZIANO							
3	RIB	0.86	±	0.18	0.02	±	0.002
4	VERTEBRA	11.25	±	0.23	1.22	±	0.39
21	RADIUS	6.51	±	3.63	0.18	±	0.07
WOOD							
1	WOOD OLD COFFIN BOTTOM FEET	0.06	±	0.05	0.04	±	0.004
2	WOOD OLD COFFIN BOTTON HEAD	2.61	±	1.31	0.06	±	0.06
7	WOOD OLD COFFIN HIGH HEADBOARD WALL + PARCHMENT	1.63	±	0.25	0.06	±	0.06
9	WOOD NEW COFFIN COVER LEFT INTERNAL HEAD	LOQ	±	-	0.03	±	0.01
10	WOOD NEW COFFIN COVER LEFT EXTERNAL HEAD	0.02	±	0.04	0.04	±	0.03
15	WOOD OLD COFFIN HIGH PART BOTTOM FEET	3.84	±	2.71	0.98	±	0.15

As, Hg y Pb (µg/g) values in Montelupo Fiorentino samples

SAMPLE	SU	BONE	As	±	σ	Hg	±	σ	Pb	±	σ
25	2089	FEMUR	1.28	±	0.12	0.31	±	0.12	94	±	9.4
26	2089	RIB	2.76	±	0.04	0.60	±	0.13	292	±	8.3
27	2089	SOIL RIB	2.46	±	0.03	0.91	±	0.32	130	±	2.08
28	2061	LARGE B.	2.50	±	0.21	1.03	±	0.43	5.6	±	0.06
29	2073	LARGE B.	2.31	±	0.18	0.51	±	0.20	169	±	5.14
30	2017	LARGE B.	1.43	±	0.25	0.27	±	0.02	11	±	0.85
31	2083	SOIL	4.55	±	0.04	0.54	±	0.17	48	±	1.28

- **Soil sample “31” Pb value minor than soil sample “27”.**
- **Soil sample“27” Pb higher value related to rib values.**
- **Femur “25” contains high Pb values .**
- **High Pb values also found in large bone child“29” SU 2073.**

VI. CONCLUSIONS AND FUTURE DEVELOPMENTS

During the development of our project many factors have contributed to obtain innovative results:

1) the proposed analytical methodology has produced a meaningful and reliable statistical analysis of our database.

2) The combination involving major elements, trace elements and REEs analysis, the strategy of sample selections and the multivariate statistic treatment of data applied to very heterogeneous and diachronic archaeological materials have provided support to develop some original methodological proposals adding new ways to overcome problems in some forensic science and forensic archaeology.

The most interesting conclusions need to be stressed:

1.Carbonized bones have been statistically differentiated from incinerated bones and **<u>class assignment of bone samples</u>** with uncertain thermal impact in dependence on their trace elements composition has shown to be feasible.

2. Chemical analysis and statistical classification of burned bones exposed to different thermal impact, **<u>are a good tool</u>**, together with analysis of bones belonging to different skeletal sectors to control diagenetic factors in order to decide whether a sample is suitable or not for forensic, biological or paleonutritional studies.

3.The use of elemental profiles found in outer bone layers (buried and cremated) for biochemical-archaeological studies **<u>is not recommended</u>**.

4. Diet profile of a population **<u>could change</u>** depending on the class of bones analyzed between the same population and individuals.

5.The analysis of heavy metals in forensic archaeological studies **<u>have been an effective tool</u>** in certain situations to rebuilt interpretations about human toxic element exposure, although an accurate strategy of sampling need to be applied.

6.Pico Della Mirandola bone samples have **<u>not presented toxic levels</u>** of As and Hg.

7.In Girolamo Benivieni corpse, some substance containing As and Hg **has probably been employed** *post-mortem* as preservative.

8.Poliziano has got a **chronic exposure to arsenic** caused by environmental factors or medical cures, but Poliziano´s death by arsenic poisoning has not been confirmed.

9.In the case of Montelupo Fiorentino individual **has not been possible** to determine whether the individual was a ceramist.

I consider the results of this thesis **an interesting contribution** to the progress of forensic methodologies. This work was born as a multidisciplinary research approach.

VI.2 Future Developments

Due to the encouraging results achieved by bone analysis, the same methodological proposals are being tested in other ancient forensic cases as studying the people died during the Spanish civil war (1936-1939) to indentify individuals. Analysis have shown to be an effective tool to know living habits of individuals excluding post-mortem contaminations.

Also comparative studies between organic and inorganic bone matter have to be developed to better understand if there are, and which are, the relationships between diagenetic processes involving both tissues employing our proposed methodology.

Eventually, non-destructive techniques like Spectral diffuse reflectance VIS-SWIR, XR Fluorescence and Laser Ablation ICP-MS, could allow to determine the mineral content of samples without any complex sample pre-treatment and facilitate the advance of future studies in some of the mentioned materials.

ACKNOWLEDGMENT

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