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Forensic Geochemistry of Bone: A Multimodal Analytical Framework for Environmental Reconstruction and PMI Estimation

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Abstract— **The accurate estimation of the post-mortem interval (PMI) and the reconstruction of the deposition environment are central challenges in forensic science. This study proposes a multimodal analytical framework integrating High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD), Ion Chromatography (IC), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to characterize both organic and inorganic components of bone. Bovine bone samples with known PMIs, exposed to various environmental conditions (surface exposure, burial, and wrapped burial), were analyzed. HPLC-DAD demonstrated high sensitivity and linearity ($R^2 > 0.99$) for quantifying citrate, revealing a strong negative correlation ($R^2 \approx 0.99$) between citrate content and the logarithm of PMI (days), corroborating and improving upon existing models. Concurrently, ICP-MS analysis detailed significant changes in the bone mineral matrix (Na, Mg, P, Sr, Ba, Fe, Cu) and revealed dynamic element transfer between the bone and surrounding soil, influenced by microbial activity, leaching, and environmental conditions. While IC provided supporting evidence, matrix effects presented challenges. The results confirm that citrate is a robust biomarker for PMI estimation and underscore the critical influence of the deposition environment on bone diagenesis. This integrated approach offers a reliable, accessible, and comprehensive protocol for forensic geochemical analysis, enhancing the interpretation of skeletal remains in both forensic and archaeological contexts.**

Keywords— **Bone diagenesis, citrate, forensic geochemistry, post-mortem interval, soil-borne interaction, HPLC -DAD, IC, and ICPMS.**

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I. INTRODUCTION

Precise quantification of both organic and inorganic constituents in bone tissue is pivotal in forensic science for estimating the post-mortem interval (PMI) and assessing skeletal preservation. Bone citrates, which play a crucial role in mineralization processes, have been studied using high-performance liquid chromatography with diode-array detection (HPLC-DAD), offering high sensitivity and specificity for separating citrate isomers in complex biological matrices [1–3]. Concurrently, the quantification of mineral elements such as calcium, phosphorus, and trace metals by inductively coupled plasma mass spectrometry (ICP-MS) provides valuable geochemical and taphonomic information [4, 5].

Although these methods have individually demonstrated efficacy in forensic applications, few studies have integrated both approaches to offer a multidimensional analysis of skeletal remains [6, 7]. This study evaluates the combined use of HPLC-DAD, ion chromatography, and ICP-MS to enhance the forensic characterization of bone samples, aiming to improve PMI estimation and environmental reconstructions

Recent studies have explored high-resolution approaches such as NMR spectroscopy, metabolomics, and shotgun metagenomics to improve PMI estimation and burial environment assessment [8, 9]. However, these advanced techniques remain costly, infrastructure-dependent, and limited in routine forensic practice [10, 11]. By contrast, the classical methods employed here remain accessible, robust, and well suited to real-world forensic contexts, particularly in the analysis of aged or fragmented skeletal material [11].

Furthermore, recent research has shown that decomposition conditions—surface vs. subsurface—significantly affect soil microbial communities, highlighting the value of combining geochemical bone analysis with environmental microbial profiling [12]. There is a recognized need for hybrid analytical frameworks that leverage both established and emerging techniques to improve PMI estimation [13].

The aim of this study was to assess the changes in citrate and mineral concentration in bovine bone samples exposed to various environmental conditions, using multiple analytical techniques including HPLC, ion chromatography (IC), and ICP-MS.

II. MATERIALS AND METHOD

A.Biological Material: Sample Collection and Preparation

1) Bone Samples

In this study, experiments were conducted on bovine cortical bone. The recovery of bones and surrounding soil was carried out in 4 April 2022.

Bovine bone remains (femur and tibia), used as the

experimental model, were obtained from local vendors in the province of Algiers. Fresh, untreated bones—free from any chemical processing—were placed in hermetically sealed ZIP-type bags and transported to the laboratory under low-temperature conditions to prevent any alteration.

Five bone samples were prepared for this investigation:

-Sample E0: Collected and stored at 0 °C immediately after the animal's slaughter. This sample served as the control (blank) for determining the initial citrate concentration (C_0).

-Sample E1: Placed on the surface, exposed to open air, and protected by a cage to prevent interference from scavengers, while maintaining exposure to natural environmental factors (heat, humidity, sunlight, rain, and wind).

-Sample E2: Buried at a depth of approximately 35 cm to assess the effect of soil on the decomposition process.

-Sample E3: Buried at the same depth but enclosed in a plastic bag, in order to evaluate the influence of the wrapping material on bone degradation.

-Sample E4: Originated from a previous experiment conducted by forensic medicine experts, dating back to 24 December 2015 (date of the cow's slaughter).

Prior to analysis, all samples were degreased according to the protocol described by Schwarcz H.P. et al., with minor modifications adapted to the laboratory conditions.

2) Soil Samples

Soil samples collected from the vicinity of the bones were placed in ZIP bags for analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), with the aim of monitoring changes in the mineral composition during the decomposition process.

A pretreatment step was necessary to ensure representative and reproducible analytical results. As soil samples contained variable moisture levels, 30 g of each sample were oven-dried at 60 °C for 24 h to standardize water content.

After drying, the samples were homogenized to improve analytical consistency, sieved to obtain a particle size below 2 mm, and subsequently ground to a fine powder before analysis.

B. HPLC-DAD Analysis: Experimental conditions

1) System performance:

High-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) analyses were performed using an Agilent 1200 series system equipped with a diode array detector. Chromatographic separation was achieved on a Zorbax SB-C18 column (150 × 4.6 mm, 5 µm particle size). The mobile phase consisted of a methanol–water mixture.

The operating conditions were as follows: the column temperature was maintained at 35 °C, the flow rate was set at 1.0 mL/min, and the injection volume was 10 µL. Isocratic elution was applied using a mobile phase composition of 5% methanol and 95% water.

The retention times of the citrate standards were closely monitored throughout the study. Analysis of the test chromatogram provided with the analytical column used for all experiments showed an average retention time of 1.32 minutes under the selected temperature and flow rate conditions.

Before the introduction of bone solutions into the column, the citrate standards exhibited an average retention time of 1.28 minutes. However, following the analysis of bone samples, this average increased to 1.42 minutes. This shift indicates that the presence of bone matrix components significantly affected the column's performance, altering the retention behavior of the citrate standards.

As previously mentioned, all sample analyses were performed both at a fixed detection wavelength of 193 nm and across a full spectral range (190–300 nm), utilizing the HPLC system's 3D wavelength scanning capabilities."

2) HPLC-DAD Method Validation Using Standard Solutions

This section evaluates the capability of the HPLC method to detect citrate in single-matrix standard solutions. Both qualitative and quantitative analyses were performed to verify that the method provided acceptable linearity across the selected calibration range (0–2.5 mM). Additionally, the standard solutions were assessed to monitor potential degradation in calibration response and linearity over time.

a). Linearity:

A total of 7 sets of standards were prepared in this study and analyzed for linearity. An $R2 > 0.98$ and a y-intercept value $\leq 10\%$ of the 2.5 mM response were considered acceptable degrees of linearity.

All analyses were performed as described in the following table:

Table I.

Peak areas and retention times for each concentration of the set of 07 STD standards.

Standard solution	Concentration (mM)	tr (min)	Peak area
STD_{00}	0,000	0,000	0,000
STD_{01}	0,100	1,346	27,600
STD_{02}	0,500	1,282	136,200
STD_{03}	1,000	1,283	273,000
STD_{04}	1,500	1,286	471,900
STD_{05}	2,000	1,287	631,800
STD_{06}	2,500	1,287	738,100

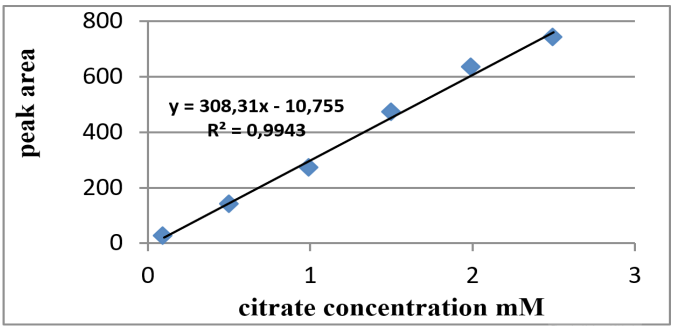


Fig 1. Typical calibration curve for the HPLC method.

All tested sets had R2 values greater than 0.99. Thus, all sets tested in this study had acceptable linearity based on the criteria defined for linearity. Furthermore, the results indicate that the use of seven concentrations [0; 0.1; 0.5; 1.0; 1.5; 2.0; 2.5 mM] of standards is sufficient to provide an acceptable linear calibration curve over a range of 0 to 2.5 mM.

b). Limits of Detection and Quantification (LOD and LOQ)

Two detection limits were determined, namely the lower limit of detection (LOD) and the lower limit of quantification (LOQ). The HPLC method had an LOD of 0.46 mM and an LOQ of 1.41 mM.

c). Instrument accuracy and precision assessment

Instrument precision, measured as the percentage relative error from the "true" value, and accuracy, measured as the percentage relative standard deviation (RSD), were evaluated by analyzing standard solutions. The instrument accuracy criterion was set at a relative error of 5%. The instrument

resolution criterion was chosen to be less than 5% RSD. The HPLC method achieved values <2% for several studies. The method has acceptable instrument accuracy and precision.

The method met the acceptance criteria for detection limit and instrument precision. The linear range of the HPLC method was determined to be 0.1mM to 2.5mM.

3) HPLC-DAD Analysis of bovine bone samples

Five (5) bovine bone samples with known PMI values were analyzed. The sample identifiers and respective PMIs are listed in Table 02. Samples were subjected to different environmental conditions: surface exposure (S 01), burial at ~30 cm depth (S 02), and burial at ~30 cm depth wrapped in a plastic bag (S 03). A fresh control sample (S 00, PMI = 0 days) and a long-term sample (S 04, PMI = 2350 days) were also included.

Table II.
Bovine bone sample information..

Sample Identifier	PMI (days)
S 00	0
S 01	60
S 02	60
S 03	60
S 04	2350

III. RESULTS AND DISCUSSION

A. PLC-DAD Analysis of citrate in bone Samples

1) Identification of citrate peaks in bone chromatograms

The chromatograms produced by the bone solutions were consistent throughout the study. In general, each chromatogram contained a large citrate peak with a retention time in the range of 1.35 to 1.40 minutes. A typical chromatogram of a sample produced in this study is shown in the figure below.

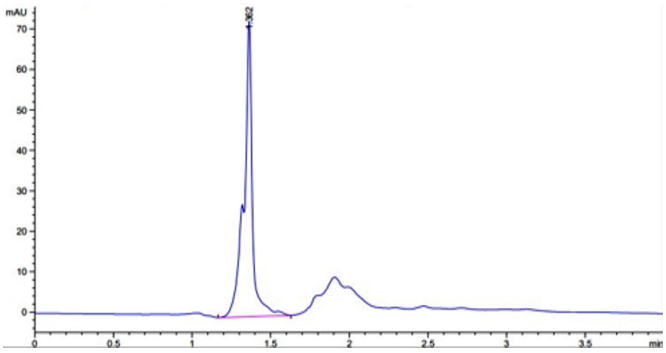


Fig 2. Chromatogram of bovine bone sample (S 02).

2) Comparison of results from samples exposed in different environmental conditions

One of the main questions raised in this study is whether different intrinsic and/or extrinsic environmental conditions (humidity, temperature, wind, soil contamination) influence citrate concentrations in bones. The data were analyzed to determine the quantitative differences in citrate concentrations between the different samples. Samples of bone placed on the surface and bone buried at a depth of approximately 30 cm, and bone buried at the same depth but after being wrapped in a plastic bag, were compared.

The chromatograms of the three types of samples were analyzed for peak separation, retention time, and peak height. Figures 02, 03, and 04 contain a representative chromatogram for each of the types of samples evaluated.

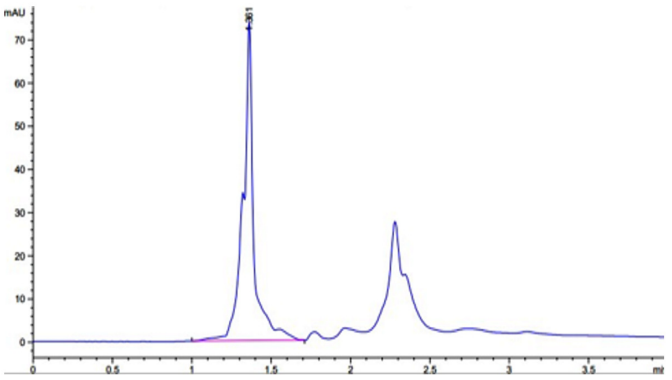


Fig 3. Chromatogram of a bone sample buried at a depth of approximately 30 cm. (S 02)

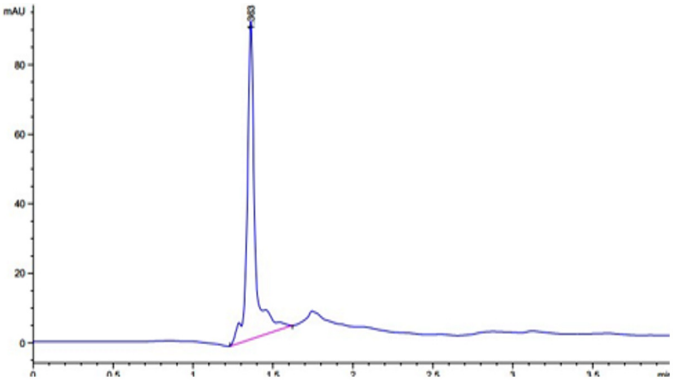


Fig 4. Chromatogram of a bone sample buried approximately 30 cm deep and wrapped in a plastic bag. (S 03).

Table III.
Citrate concentrations and estimated PMI for the samples analyzed.

Sample	Concentration (mM)	concentration (Wt %)
S 00	1.180	0.740
S 01	1,110	0,700
S 02	1,010	0,640
S 03	1,150	0,720

3) Etxperimental Evidence of citrate reduction in buried bone

The supernatant from each sample digestion was analyzed by HPLC and the citrate content (% by weight) was determined. The data on the compound citrate content were plotted against the IPM (log t) as shown in Figure 05. In general, a direct relationship was found between the citrate content in the bone samples and the IPM. The results obtained are very good and are similar to those obtained by Schwarcz et al.

Table VI.
Results for buried samples.standards.

sample	Concentration (mM)	Log(T)(days)	Concentration (Wt (%)
S 00	1,180	0.000	0,740
S 02	1,010	1,778	0,640
S 04	0,910	3,370	0,570
Average	1,033	1,716	0,650
SD	0,111	1.686	0.085
RSD (%)	13.200	98.300	13.100

N.B: In addition to sample Ech 00, samples buried under the same environmental conditions, Ech 02 and Ech 04, were selected in order to study the relationship between the decrease in citrate content and the IPM.

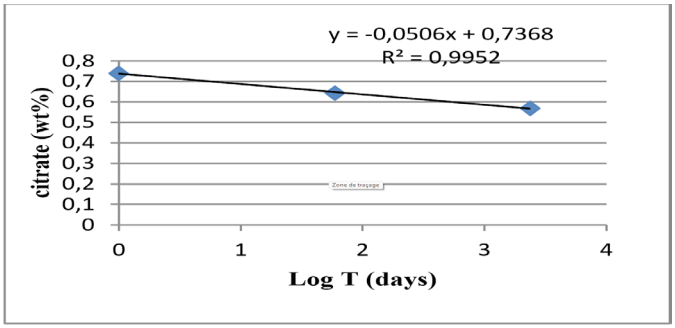


Fig 5. Graph showing citrate content (% by weight) as a function of log T (days) for three bovine bone samples.

4) Comparison with published results

There is a direct correlation between citrate content and IPM (days) for our data (Fig. 05). The R2 value was approximately 0.99, which is better than the R2 = 0.98 value reported by Schwarcz et al. when citrate content was plotted against log t (t = days), as shown in (Fig. 06).

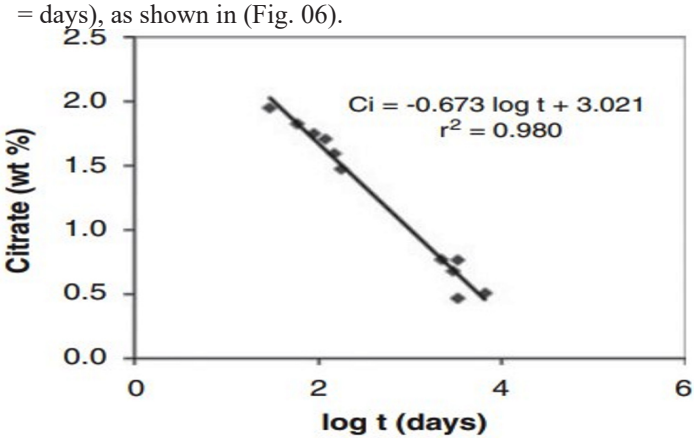


Fig 6. Plot of citrate content (% by weight) versus log t (days) adapted from Schwarcz et al. 2010.

The ideal R2 value for calibration is generally greater than 0.980, but for this type of comparison, 0.980 is actually very good. However, the small number of samples analyzed in this study indicates that further research by multiple laboratories is needed to fully validate the method.

B. Ion chromatography (IC) Results

1) Ion chromatography (IC) analytical conditions

Anion analysis was performed using a Dionex ICS-3000 ion chromatography system (Thermo Fisher Scientific, Sunnyvale, CA, USA). The system was equipped with an autosapler ,a conductivity detector and operated according to the manufacturer's recommendations.

Separation was achieved on a HIPIC AG 12A column (150 × 4.6 mm, 5 µm particle size). Isocratic elution was employed.

The operating conditions were as follows: a flow rate of 1.5 mL/min and an injection volume of 50 µL.

2) Ion chromatography (IC) Method Validation Using Standard solutions

For the quantitative determination of citrate in bone (see Appendices B and C) and following the protocol mentioned above, we designed the calibration curve for the citrate solutions that were prepared and analyzed by IC. The results obtained are presented in the following table:

Table V.

Peak air and retention time for each concentration of the 06 STD standard set.

Standard solution	Concentration (mM)	The Air of Pic	Retention time(min)
STD_{00}	0,000	0000	0.000
STD_{01}	0,100	1183	4,330
STD_{02}	0,500	2258	4,287
STD_{03}	1,000	3022	4,350
STD_{04}	1,500	5299	4,340
STD_{05}	2,000	6673	4,497
STD_{06}	2,500	8567	4,267
Average (STD01-STD06)	1,267	4500	4,345

A calibration curve was plotted using the results obtained in the table above.

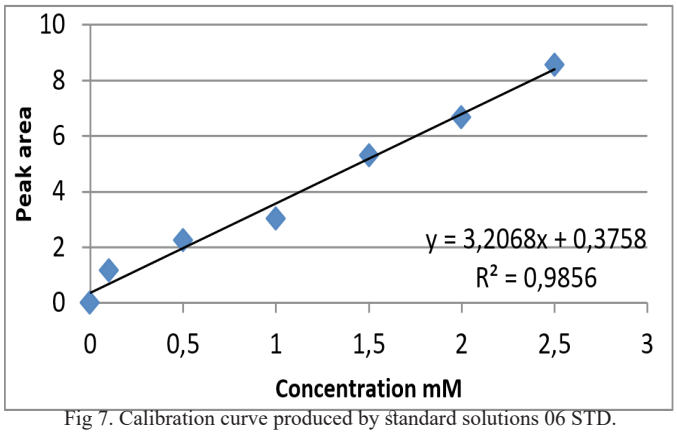


Fig 7. Calibration curve produced by standard solutions 06 STD.

Table VI.

peak air and retention time for each concentration of all 05 samples

Standard solution	Retention time (min)	The air of pic	Concentration of citrate (mM)	Retention time (min)
S00	3,613	1702,530	530,790	3,613
S01	3,683	1695,230	528,520	3,683
S02	-	-	-	-
S03	3,700	1405,489	438,167	3,700
S04	3,640	1605,734	500,610	3,640

After processing the results and comparing them with the reference samples, it was found that the tr of the unknown peak is similar to the tr of the reference samples. We also found that the peak area is very large compared to the reference samples, indicating that this peak may represent citrate and that the reason for the difference in tr is due to the intensity of the citrate concentration in the bone samples.

Based on the results obtained and due to time constraints, we were unable to modify the protocol in order to repeat the previous analyses and obtain better results.

Note: We did not obtain any results for sample E2 due to the almost non-existent amount of extract prepared, which meant we were unable to inject it.

HPLC analysis demonstrated a robust and reliable method for detecting and quantifying citrate. The linearity of the system was confirmed by an excellent correlation ($R^2 > 0.99$) between standard concentrations and chromatographic peak areas. The limits of detection (LOD = 0.46 mM) and quantification (LOQ = 1.41 mM), along with instrumental precision (errors < 2%), confirm the validity of this method for concentrations ranging from 0.1 mM to 2.5 mM. However, the introduction of bone solutions slightly altered retention times, highlighting the influence of biological matrices on chromatographic performance.

Analysis of bone samples revealed a progressive decrease in citrate content (% by weight) over postmortem interval (PMI), particularly in buried samples. This decrease is consistent with the findings of Schwarcz et al. (2010), with a correlation coefficient of approximately $R^2 \approx 0.99$ for the curve of log(time) versus citrate content, validating citrate as a reliable temporal marker in bone tissue.

A strong linear correlation was observed ($R^2 \approx 0.99$) between citrate content (% by weight) over PMI interval (Figure 05) particularly in buried samples. This decrease is consistent with, the finding of Schwarcz et al. [14]. This confirms citrate as a reliable biomarker for PMI estimation.

Analysis revealed a progressive decrease in citrate concentration with increasing PMI, particularly in buried samples (S 02, S 04). The citrate content (% weight) was plotted against the logarithm of PMI (log t(days)) for samples S 00, S 02, and S 04 (Table VII).

Table VII.
Citrate content in bones with Log (PMI)

Sample	[Citrate] (mM)	[Citrate] (Wt %)	Log (PMI) (days)
S 00	1.180	0.740	0.000
S 02	1.010	0.640	1.780
S 04	0.910	0.570	3.370

(Figure 05: Plot of citrate content (% weight) vs. log t (days) for buried bovine bone samples.).

C. Analysis by ICP-MS:Experimental conditions

Elemental analysis was carried out using an inductively coupled plasma mass spectrometer (ICP–MS, Thermo Fisher Scientific, USA). The instrument was operated following the manufacturer's recommended operating conditions. High-purity argon (N60, 99.9999%) was used as both the plasma and carrier gas, while high-purity helium served as the collision gas to reduce spectral interferences.

The plasma exhaust flow rate was maintained between 67 and 90 $m^3\ h^{-1}$. The cooling system operated with a water flow rate higher than 5.5 L min^{-1}, at a temperature ranging from 20 to 30 °C (maintained at 21 °C), and a pressure between 0.25 and 0.60 MPa. The cooling water quality met the manufacturer's specifications, with a pH of 6.8, electrical conductivity below 1000 µS cm^{-1}, and suspended solid particles smaller than 50 µm. The argon gas supply pressure was maintained at approximately 6 bar throughout the analysis.

1) The mineral components of bones

In order to identify and monitor changes occurring in the mineral components of bones, and after preparing the control sample for comparison with the other samples in accordance with the protocol described in section 5.1.2, quantitative and qualitative analysis of this sample yielded the results shown in the following figure.

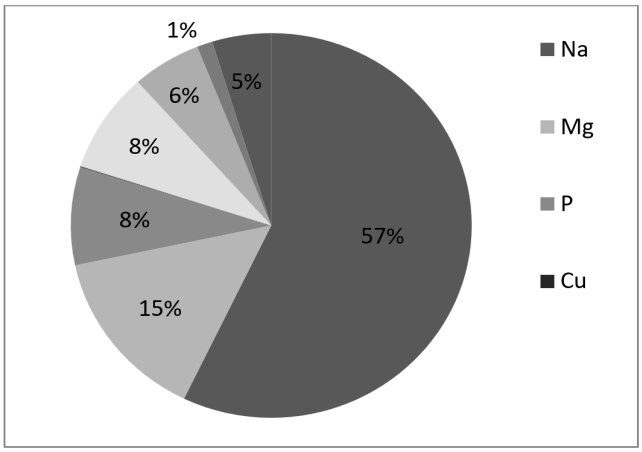


Fig 8. The mineral components of bones.

After analyzing sample E0, we find that the mineral composition contains numerous mineral elements, foremost among which is sodium at 57%, followed by magnesium at 15%, phosphorus and iron sharing an equal total of 16%, then barium at 6% and strontium with the lowest percentage, estimated at 1%. The other matrices account for 5%.

2) The evolution of the mineral components of bones:

As previously indicated in section 5.2, a total of 5 bone samples were prepared in order to determine their mineral composition and study the changes that occur over time due to natural factors selected for each sample. The results obtained are presented in the table below:

The afore mentioned protocol was followed to analyze soil samples (S01, S02, S03) before placing the bones and after their recovery (S01-01, S02-01, S03-01) to demonstrate the changes.

Table VIII.
Changes in the mineral components of bones in mg/Kg

	Na	Mg	P	Cu	Sr	Ba	Fe
E_0	48934.590	12414.766	7065.812	54.789	7200.854	4774.406	1.079.375
E_1	47372.890	9707.471	6273.123	108.808	6701.899	15218.271	1.126.958
E_2	40815.134	8300.785	5975.574	76.236	4634.801	14799.681	2.104.448
E_3	48130.706	8861.153	6619.489	93.356	5079.584	16474.039	950.502

The following histogram shows the results obtained after ICP-MS analysis of bone samples.

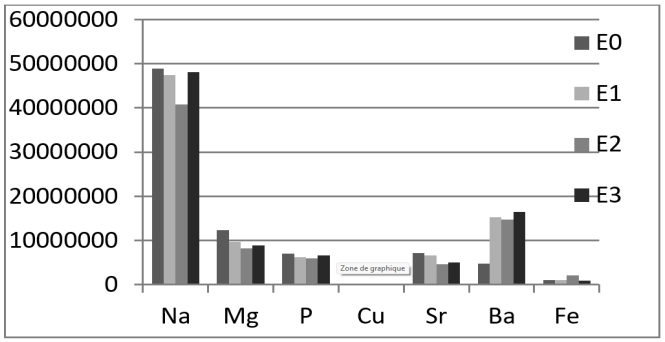


Fig 9. Evolution of the mineral components of the samples (mg/Kg).

The afore mentioned protocol was followed to analyze soil samples (S01, S02, S03) before placing the bones and after their recovery (S01-01, S02-01, S03-01) to demonstrate the changes in mineral composition occurring on the bones. The results are listed in the table below:

The following histograms represent the results obtained after ICP-MS analysis of soil samples (S01, S01-01; S02, S02-01, and S03, S03-01) before and after the addition of bone samples.

Sample S01:

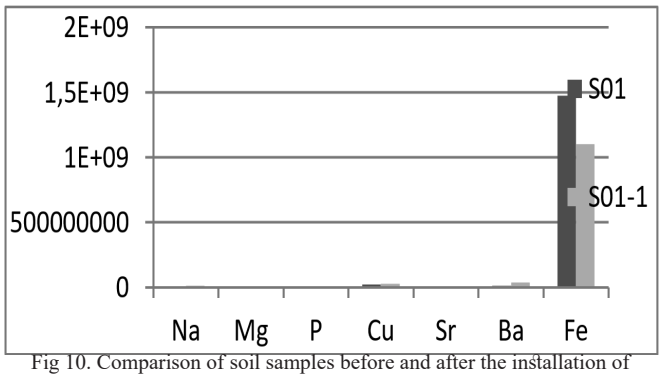


Fig 10. Comparison of soil samples before and after the installation of sampleE01(mg/kg)

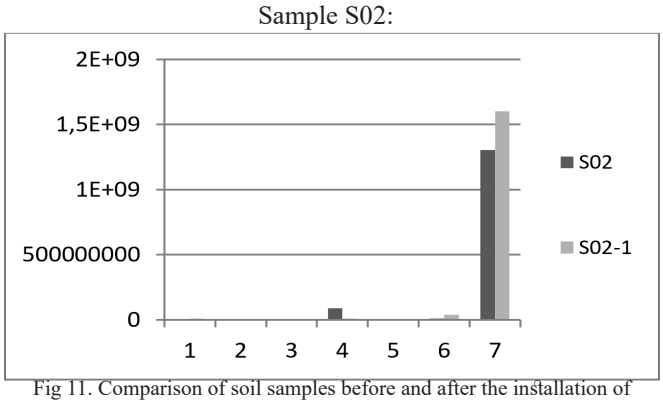


Fig 11. Comparison of soil samples before and after the installation of sample E02 in mg/kg.

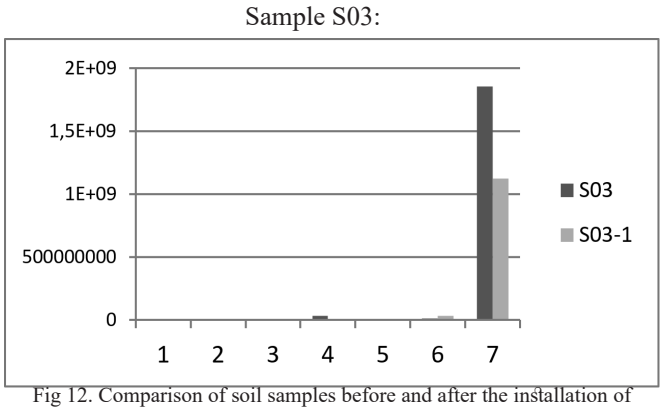


Fig 12. Comparison of soil samples before and after the installation of sample E03 in mg/kg.

For sample E1:

Table IX.
Comparison of changes in mineral composition (in mg/kg) between bone sample E1 and the surrounding soil where it was placed.

	Na	Mg	P	Cu	Sr	Ba	Fe
E1-E0(%)	-3,19	-21,81	-11,22	98,59	-6,93	218,75	4,41
S01-1-S01(%)	108,58	-29,50	0,00	49,80	-98,29	230,82	-25,44

* The graph shows that the concentration of Na has decreased in the bone samples, which corresponds to an increase in its concentration compared to the soil sample. The logical explanation is the possibility of transferring a quantity of this element from the bone to the soil, or that the sodium in the soil comes from either irrigation water or groundwater.

* As for Fe, there is an increase in its concentration in the bone sample and a decrease in the soil, which is due to the transfer of this element from the soil to the bones.

* As for Ba and Cu, the concentration of these two elements increased in both bone and soil samples, and this is the result of the activity of microorganisms present in the soil.

* For magnesium, the loss of this element in both matrices (E1 and S01) occurs due to leaching, absorption by microorganisms, low retention of soil cations, and precipitation of secondary minerals.

* For strontium, there is a decrease in its percentage in bone and soil because it dissolves in water, and therefore it is likely to move deeper into the soil and reach groundwater.

* Phosphorus is an essential and critical nutrient for plant mineral nutrition. Due to its low concentration in bone and low solubility, there is a decrease in the bone sample, but it remains stable in the soil sample.

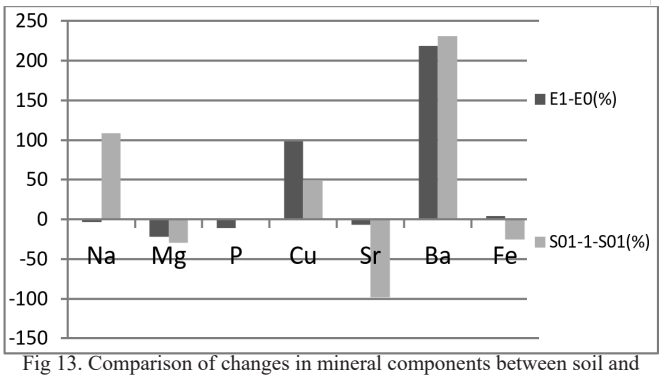


Fig 13. Comparison of changes in mineral components between soil and bone.

For sample E2:

Table X.
Comparison of changes in mineral composition (in mg/kg) between bone sample E2 and the surrounding soil where it was placed.

	Na	Mg	P	Cu	Sr	Ba	Fe
E2-E0(%)	-16,59	-33,14	-15,43	39,14	-35,64	209,98	94,97
S02-1-S02(%)	298,08	5,49	30,04	87,13	-6,76	283,64	22,56

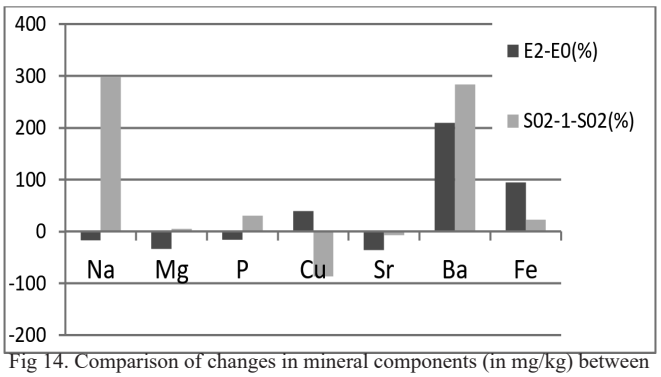


Fig 14. Comparison of changes in mineral components (in mg/kg) between soil and bone.

*The results of the analysis of samples E2 and S2 revealed an increase in the proportion of Cu and Ba in both matrices. This increase is due to the phenomenon mentioned above. The increase in Fe is due to iron deposition in the soil, as the bone sample was buried at a specific depth in the soil.

*For strontium, there is a decrease in its percentage in both the bone and the soil because it dissolves in water, and therefore it is likely to move deeper into the soil and reach the groundwater.

*As for Na, P, and Mg, their percentage decreases in the bone sample with an increase in the soil, and this is due to the transfer of these elements from the bone to the soil.

For sample E3:

Table XI.
Comparison of changes in mineral composition between bone sample E3 and the surrounding soil where it was placed.

	Na	Mg	P	Cu	Sr	Ba	Fe
E3-E0(%)	-1,64	-28,62	-6,32	70,39	-29,46	245,05	-11,94
S03-1-S03(%)	17,28	-43,38	-52,14	-75,46	436,53	166,67	-39,43

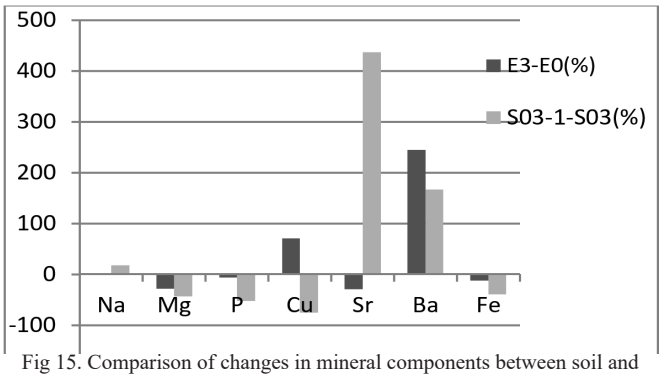


Fig 15. Comparison of changes in mineral components between soil and bone.

Table XII.
ICP-MS results for bone samples (concentrations in counts).

Sample	Na	Mg	P	Cu	Sr	Ba	Fe
S 00	48934,590	12414,766	7065,812	54,789	7200,854	4,774,406	1,079,375
S 01	47372,890	9707,471	6273,123	108,808	6701,899	15,218,271	1,126,958
S 02	40815,134	8300,785	5975,574	76,236	4634,801	14,799,681	2,104,448
S 03	48130,706	8861,153	6619,489	93,356	5079,584	16,474,039	950,502

* Extrapolating from the results obtained after analyzing sample E3, an increase in the percentage of Sr was observed in the soil sample with a decrease in its percentage in the bone sample, and this is the result of its deposition on the surface of the waterproof plastic bag used to wrap the bone sample before burial.

* As for Ba, the concentration of this element increased in both the bone samples and the soil samples, and this is the result of the activity of microorganisms present in the soil.

* Sodium in the soil comes either from irrigation water or groundwater. The percentage of sodium in the soil may increase due to the deposition of calcium and magnesium from the soil in the form of poorly soluble compounds such as lime and gypsum, which explains their increase in the soil sample.

* We explain the lack of iron (Fe) in the soil sample by its absorption by plants, as it is a major component for plant photosynthesis and acts as a cofactor for many enzymes necessary for plant hormone synthesis. The decrease in its percentage in the bone sample is due to the activity of microorganisms.

Ion chromatography (IC) confirmed the results obtained by HPLC, despite some limitations (e.g., lack of data for sample E2). The retention times of the suspected peaks in bone samples matched those of citrate standards, supporting its identification.

ICP-MS analysis provided additional insight into the evolution of the bone's mineral components and their interactions with the surrounding soil. Notable transfers of elements such as sodium (Na), iron (Fe), barium (Ba), and copper (Cu) were observed between bone and soil, influenced by environmental factors (humidity, microbial activity, leaching). These mineral changes confirm that the environment plays a key role in both the degradation of bone and the mobilization of elements, alongside organic degradation.

3) Ion Chromatography (IC) Analysis

IC results supported the HPLC findings, with peaks in bone samples exhibiting retention times similar to citrate standards. The large peak areas in bone samples suggested high citrate concentration. However, data for sample S 02 was unavailable due to insufficient extract volume, and matrix effects caused some variability, indicating a need for method optimization for complex biological matrices.

4) ICP-MS Mineral Analysis

ICP-MS analysis revealed significant changes in the bone mineral matrix and dynamic interactions with the soil environment.

5) Bone Mineral Evolution

The control sample (S 00) was primarily composed of Na (57%), Mg (15%), P and Fe (16% combined), Ba (6%), and Sr (1%). Comparative analysis showed a decrease in Na, Mg, P, and Sr concentrations in weathered samples (S 01, S 02, S 03), while Ba, Cu, and Fe concentrations increased (Table XII).

6) Changes in Bone Mineral Composition during Burial

Analysis of soil samples before and after bone deposition confirmed significant elemental transfer:

-Sodium (Na): Decreased in bone but increased in soil, suggesting leaching from bone into the soil or contribution from external sources like groundwater.

- **Iron (Fe)**: Increased in buried bones (S02)، indicating uptake from the soil, while its decrease in some soils suggested plant uptake.

- **Barium (Ba) & Copper (Cu)**: Marked increases in both bone and soil, likely due to microbial activity and redistribution.

Strontium (Sr): Decreased in both matrices due to its high solubility and leaching into groundwater.

- **Magnesium (Mg) & Phosphorus (P)**: Decreased in bone due to leaching and microbial consumption. P remained stable in some soils due to low solubility and high nutrient demand.

- **Sample E3 (Wrapped):** The plastic wrap altered exchange dynamics. A significant increase of Sr in the soil was observed, likely due to its deposition on the bag's surface, preventing reintegration into the bone.

These results highlight the complex geochemical processes—including leaching, microbial mediation, cation exchange, and precipitation—that govern bone diagenesis and must be considered for accurate environmental reconstruction.

IV. CONCLUSION

This study demonstrates the reliability and relevance of classical biochemical approaches while highlighting the added value of a multimodal analytical framework for forensic bone investigation. The validation of HPLC-DAD as a robust method for quantifying citrate confirms its strong potential as a temporal marker, given the clear correlation observed between citrate depletion and PMI. Complementarily, ICP-MS provided critical insights into the geochemical interactions between bone and burial environments, revealing significant elemental mobility that enhances environmental reconstruction. Although IC delivered supportive data, its sensitivity to matrix effects indicates the need for further optimization.

Overall, the integration of organic and inorganic analyses emerges as the most effective strategy for PMI estimation and taphonomic interpretation, offering a comprehensive and operationally accessible protocol for routine forensic casework.

Aligns with the broader evidence that assessing citrate and metal concentrations remains a robust, cost-effective, and widely applicable method, particularly in contexts with limited access to advanced technologies. As previously highlighted by

Pilli et al. (18–21), emerging molecular approaches—such as

DNA degradation profiling or epigenetic markers—open promising avenues to further refine post-mortem dating.

Thus, the present study reinforces the reliability of established biochemical methods while acknowledging, in line with Pilli et al., the potential of complementary molecular techniques. It advocates for a pragmatic and integrative perspective to enhance both the accuracy and the applicability.

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