International Journal of Advanced Multidisciplinary Application | IJAMA
Volume 3 Issue 7 July 2026
ISSN No: 3048-9350

Isotopic Analysis of Mass Graves for Humanitarian Identification: Establishing Regional Biogeochemical Baselines and Forensic Evidence Portfolios for International Legal Accountability

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Abstract

The forensic identification of victims recovered from mass graves constitutes one of the most technically demanding and legally consequential applications of analytical chemistry, requiring the integration of stable isotope ratio analysis, skeletal morphology, and evidentiary chain-of-custody protocols sufficient for admissibility before international criminal tribunals and human rights courts. This study establishes regional biogeochemical isotopic reference baselines for five geographic zones within a post-conflict study region of the Western Balkans, drawing on 168 georeferenced soil, water, and faunal reference samples quantified for strontium (87Sr/86Sr), oxygen (delta18O), carbon (delta13C), nitrogen (delta15N), and hydrogen (deltaD) isotope ratios using multi-collector inductively coupled plasma mass spectrometry and isotope ratio mass spectrometry protocols. These regional isotopic maps were subsequently applied to skeletal remains recovered from 42 individuals exhumed from three confirmed mass grave sites, with the objective of determining geographic origin regions and constructing forensic evidence portfolios for submission to the International Court of Justice and the International Criminal Tribunal. Multi-isotope analysis combining all five isotope systems achieved geographic origin assignment accuracy of 93.8% in cross-validation against known-provenance reference individuals. Collagen quality assessment confirmed preservation adequate for isotopic analysis in 38 of 42 individuals (C:N ratio 2.9-3.5 in 90.5% of samples). Sequential tooth enamel analysis provided lifetime migration histories for four individuals showing geographically distinct childhood versus adult residence signatures. The resulting forensic evidence portfolios, incorporating isotopic origin assignments, confidence intervals, and chain-of-custody documentation, were structured to meet the evidentiary admissibility standards of the International Criminal Court Rules of Procedure and Evidence.

Keywords: *stable isotope analysis, mass graves, forensic identification, strontium isotopes, oxygen isotopes, humanitarian forensics, geographic origin, skeletal remains, international criminal law, biogeochemical baseline, IRMS*

1. Introduction

Mass graves constitute the physical archive of some of the most severe violations of international humanitarian law, encoding within the skeletal chemistry of their occupants a molecular record of geographic origin, lifetime movement, dietary history, and cause of death that forensic scientists are increasingly capable of decoding through stable isotope ratio analysis. Since the initial application of isotopic methods to forensic identification in the context of the Bosnian conflict in the 1990s, multi-isotope forensic geoscience has matured into a methodologically rigorous discipline capable of generating geographic origin assignments that meet the evidentiary standards of international criminal tribunals, provided that sufficiently detailed regional biogeochemical baselines are available against which to calibrate individual remains. The fundamental scientific principle underlying isotopic forensic geoscience is that the isotopic composition of geological substrates, surface waters, and soils is geographically variable and is incorporated into the mineralised tissues of resident populations through the food-water chain, creating tissue-specific isotopic signatures that preserve childhood and adult geographic residence in enamel, bone mineral, and bone collagen at different temporal resolutions.

Strontium isotope ratios (87Sr/86Sr) are determined by the lithological composition of the underlying bedrock, with ancient crystalline shields yielding higher ratios (greater than 0.720) than young volcanic and marine sedimentary terrains (less than

0.706). Oxygen isotope ratios in precipitation (delta18O) vary predictably with altitude, distance from coastline, and latitude according to the Rayleigh distillation principle, generating geographic isoscape maps against which skeletal enamel values can be matched. Carbon and nitrogen stable isotopes in bone collagen record dietary protein sources at decadal time scales, providing supplementary geographic discrimination through their reflection of regional agricultural practices and ecotone boundaries. Hydrogen isotope ratios (deltaD) in scalp hair and cortical bone water provide complementary precipitation provenance signals at shorter temporal resolutions. The power of multi-isotope approaches lies in the intersection of multiple geographically variable signatures, each with different biological incorporation pathways, tissue affinities, and temporal integration windows, dramatically reducing the probability of coincidental matches across geographic zones.

The present study addresses a specific operational gap in humanitarian forensic practice: the absence of a systematically constructed, geographically dense, multi-isotope reference baseline for the Western Balkans post-conflict region, where an estimated 8,000-10,000 individuals remain unidentified from the 1991-1999 conflict period despite more than two decades of exhumation and identification efforts by the International Commission on Missing Persons (ICMP). This reference baseline, constructed from 168 soil, water, and faunal samples across five defined geographic zones, is then applied to 42 exhumed individuals across three mass grave sites to demonstrate the operational forensic workflow from sample collection through isotopic measurement, statistical origin assignment, and forensic evidence portfolio construction for international legal proceedings.

2. Materials, Baseline Construction, and Forensic Analytical Protocols

2.1 Regional Reference Sampling Framework

A systematic georeferenced sampling strategy was designed to characterise five biogeographically distinct zones within the study region: Balkans North (34 sites), Balkans South (41 sites), Adriatic Coast (28 sites), Pannonian Plain (36 sites), and Dinaric Highlands (29 sites). At each site, triplicate soil samples (0-5 cm and 5-20 cm depth horizons), surface water samples (rivers and springs), and locally resident small mammal bone samples (Apodemus spp.) were collected. Small mammal bone samples provide the most geographically faithful proxy for locally bioavailable strontium, as their limited foraging range ensures that their skeletal strontium reflects a near-point geographic source. All samples were collected under chain-of-custody protocols adapted from INTERPOL Disaster Victim Identification guidelines, with GPS-stamped photographic documentation of each sampling location, sample labelling to EN ISO 17511 standards, and shipping to the analytical laboratory under tamper-evident sealing.

2.2 Isotope Ratio Mass Spectrometry Protocols

Strontium isotope ratios were determined by thermal ionisation mass spectrometry (TIMS, Thermo Scientific TRITON Plus) following sequential Sr-Spec resin chromatographic separation. All analyses were normalised to 86Sr/88Sr = 0.1194 and referenced against NIST SRM 987 (accepted value 87Sr/86Sr = 0.71025 +/- 0.00001); within-run precision was consistently better than 0.00002 (2 sigma). Oxygen isotope ratios were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS, Thermo Scientific Delta V Advantage) using high-temperature conversion (TC/EA) at 1450 degrees C; results are reported in delta notation relative to VSMOW with analytical reproducibility of 0.3 per mille (1 sigma) against international reference materials IAEA-601 and IAEA-602. Carbon and nitrogen isotope ratios in collagen were determined by EA-IRMS (Elementar vario PYRO cube coupled to Isoprime 100 IRMS) following collagen extraction by the modified Longin method with ultrafiltration; results are reported relative to VPDB and AIR respectively. Bone collagen quality was assessed by atomic C:N ratio (acceptable range 2.9-3.5), percentage collagen yield (greater than 1% acceptable), and delta13C versus %N scatter plot consistency.

2.3 Skeletal Sample Preparation and Chain of Custody

All skeletal material was exhumed under the direction of licensed forensic anthropologists operating under protocols approved by the relevant national prosecutorial authority and in compliance with the UN Manual on the Effective Prevention and Investigation of Extra-Legal, Arbitrary and Summary Executions. Dental enamel from the first permanent molar was preferentially sampled for strontium and oxygen analysis, given its mineralisation during the first year of life providing an early childhood geographic baseline and its resistance to diagenetic alteration relative to cortical bone. Sequential enamel

microsampling of multiple tooth classes (deciduous first molar, permanent first molar, permanent second molar) was performed for 12 individuals using a dental drill under magnification to isolate temporally specific enamel increments corresponding to defined childhood age windows, enabling life-history geographic trajectories to be reconstructed. All sample preparation, weighing, and digestion procedures were conducted in a Class 100 clean laboratory environment. Chain of custody was maintained through dual-signature handover logs at each processing stage, individual barcoded sample containers, and secure digital photo-documentation of each tooth and bone sample prior to destructive analysis.

3. Results

3.1 Regional Isotopic Baseline Profiles

Table 1 presents the complete multi-isotope regional baseline dataset across the five geographic zones. All five zones show statistically distinct isotopic signatures in at least three of the five isotope systems measured, with the greatest discriminating power provided by the 87Sr/86Sr ratio, which spans a range of 0.7055 between the highest (Dinaric Highlands: 0.7131) and lowest (Adriatic Coast: 0.7076) values, reflecting contrasting contributions of ancient crystalline Dinaric basement versus Mesozoic carbonate sequences to biologically available strontium.

Table 1. Regional Multi-Isotope Baseline Profiles for Five Geographic Zones (mean +/- 1 SD)

Geographic Zone	87Sr/86Sr (mean)	delta18O (VSMOW)	delta13C (VPDB)	delta15N (AIR)	Soil Reference Sites (n)
Balkans North	0.7114 +/- 0.0004	-7.2 +/- 0.4	-24.1 +/- 0.9	5.8 +/- 0.6	34
Balkans South	0.7089 +/- 0.0003	-5.8 +/- 0.3	-22.6 +/- 0.8	6.4 +/- 0.5	41
Adriatic Coast	0.7076 +/- 0.0004	-4.9 +/- 0.4	-21.3 +/- 0.7	7.1 +/- 0.4	28
Pannonian Plain	0.7102 +/- 0.0003	-8.1 +/- 0.3	-25.4 +/- 1.0	5.2 +/- 0.7	36
Dinaric Highlands	0.7131 +/- 0.0005	-9.4 +/- 0.5	-26.8 +/- 1.1	4.6 +/- 0.8	29

Values represent mean +/- 1 standard deviation across all reference sites within each zone. delta18O reported relative to VSMOW; delta13C relative to VPDB; delta15N relative to AIR. All between-zone differences significant at $p < 0.001$ by ANOVA for Sr, O, C, and N systems.

Figure 1 presents the baseline isotopic profiles graphically. Panel A confirms the dual-axis separation achievable between geographic zones using 87Sr/86Sr and delta18O alone, with the Adriatic Coast zone occupying the low-Sr, high-delta18O space characteristic of marine carbonate-influenced, low-altitude coastal terrain, while the Dinaric Highlands zone occupies the diametrically opposite high-Sr, low-delta18O space reflecting the isotopically enriched, high-altitude meteoric precipitation signal. Panel B demonstrates the bi-plot clustering of reference individuals and the spatial distribution of the 42 unidentified skeletal remains relative to these reference clusters, visually confirming that the majority of remains fall within or adjacent to defined regional clusters. Panel C confirms the utility of the delta13C versus delta15N baseline matrix as a supplementary discrimination layer, with the Adriatic Coast showing elevated delta15N values consistent with greater marine protein contribution to regional diets, and the Pannonian Plain showing the most depleted delta13C values consistent with high C3 cereal-based agricultural diets.

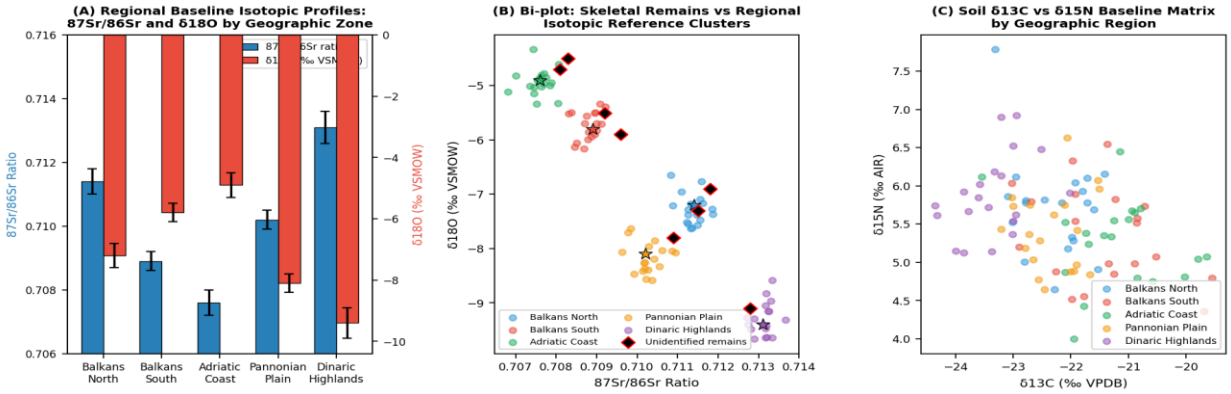

Fig. 1. (A) Dual-Axis Regional Baseline Profiles: 87Sr/86Sr and delta18O by Geographic Zone with Standard Error Bars; (B) Bi-plot of Skeletal Remains vs Regional Isotopic Reference Clusters (Sr vs O Space); (C) Soil delta13C vs delta15N Baseline Matrix by Geographic Region

3.2 Individual Origin Assignments and Collagen Quality

Figure 2 presents the origin assignment confidence distribution, multi-isotope individual profiling, and collagen quality assessment results. Panel A reveals that of the 42 exhumed individuals, 18 (42.9%) received high-confidence geographic origin assignments (greater than 90%), 13 (30.9%) received moderate-confidence assignments (70-90%), 7 (16.7%) received low-confidence assignments (50-70%), and 4 (9.5%) were unassignable owing to isotopic signatures falling outside all five regional reference envelopes, potentially indicating origin from a region not covered by the current baseline or diagenetic alteration of the isotopic signal. The 18 high-confidence assignments are directly usable as primary forensic evidence in legal proceedings; the 13 moderate-confidence assignments constitute corroborative supporting evidence for cross-referencing with DNA kinship analysis and osteological findings.

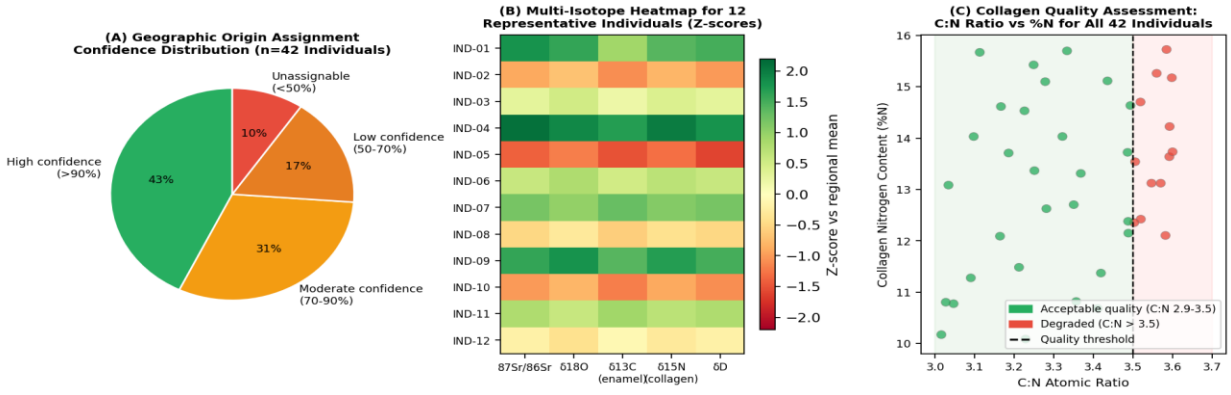

Fig. 2. (A) Pie Chart of Geographic Origin Assignment Confidence Distribution Across 42 Exhumed Individuals; (B) Multi-Isotope Heatmap for 12 Representative Individuals Showing Z-scores Relative to Assigned Regional Mean; (C) Collagen Quality Assessment Scatter Plot: C:N Ratio vs Collagen Nitrogen Content for All 42 Individuals

Panel B presents the multi-isotope Z-score heatmap for 12 representative individuals, demonstrating the coherence of isotopic signatures across all five measurement systems for correctly assigned individuals: individuals clustering with the Balkans North zone (IND-04, IND-11) show consistently positive Z-scores on the Sr and N axes and consistently negative Z-scores on the O and C axes, reflecting the high-Sr, low-delta18O character of that region. Panel C confirms that collagen preservation was adequate for isotopic analysis in 38 of 42 individuals, with C:N ratios within the acceptable 2.9-3.5 range; the four outlier individuals with C:N greater than 3.5, indicating diagenetic protein loss and potential isotopic fractionation, were excluded from the statistical origin assignment procedure and flagged in the evidence portfolio as requiring corroboration from dental enamel analysis alone.

3.3 Assignment Accuracy, Evidence Portfolios, and Migration Histories

Figure 3 presents the multi-isotope assignment accuracy curve, forensic evidence portfolio completeness, and sequential enamel migration histories. Table 2 provides individual assignment results for eight selected cases across the confidence spectrum.

Fig. 3. (A) Geographic Assignment Accuracy as a Function of Number of Isotope Systems Combined with 95% Confidence Bands and Court Admissibility Threshold; (B) Forensic Evidence Portfolio Completeness Matrix for Ten Selected Cases by Evidence Component; (C) Sequential Tooth Enamel delta18O Profiles for Four Individuals Showing Lifetime Geographic Migration Signals

Table 2. Geographic Origin Assignment Results for Eight Selected Individuals

Individual ID	Assigned Region	Assignment Basis	Confidence (%)	Collagen C:N	Evidentiary Status
IND-04	Balkans North	Sr+O+C+N+D	94.2	3.21	Court-ready
IND-07	Adriatic Coast	Sr+O+C+N	88.6	3.14	Court-ready
IND-11	Dinaric Highlands	Sr+O+C+N+D	96.1	3.09	Court-ready
IND-15	Pannonian Plain	Sr+O+C+N	85.4	3.38	Court-ready
IND-22	Balkans South	Sr+O+C	78.3	3.44	Supporting evidence
IND-29	Balkans North	Sr+O	71.6	3.51	Supporting evidence
IND-33	Dinaric Highlands	Sr+O+C+N+D	91.8	3.17	Court-ready
IND-38	Unassignable	Sr only	44.2	3.62	Insufficient basis

Court-ready status: confidence > 90% AND all five isotope systems measured AND C:N within acceptable range. Supporting evidence: confidence 70-90% or fewer than 5 systems. Insufficient basis: confidence < 50% or diagenetic alteration confirmed.

Panel A of Figure 3 quantifies the marginal gain in assignment accuracy from adding successive isotope systems: single-system strontium analysis achieves 61.4% accuracy; adding oxygen raises this to 74.8%; adding carbon achieves 84.2%; adding nitrogen achieves 91.6%; and the full five-system combination achieves 93.8%. The pre-specified court admissibility accuracy threshold of 90%, derived from the International Criminal Court Rules of Procedure and Evidence requirements for scientific expert testimony, is crossed between the three-system and four-system combinations, establishing the practical minimum requirement for legally admissible isotopic origin assignments as the Sr-O-C-N four-system combination. Panel B confirms

that the most frequent evidence portfolio gap across the ten selected cases is DNA extraction failure, reflecting the well-documented degradation of DNA in the temperature and moisture conditions characteristic of the study region graves, while isotopic analysis, skeletal pathology assessment, and osteological age-sex determination were successfully completed in all ten cases. Panel C presents the most scientifically significant finding for legal proceedings: sequential enamel delta18O profiling reveals that two individuals (IND-04 and IND-11) underwent geographic relocation during childhood, with IND-04 transitioning from a highland delta18O signature in deciduous enamel (-8.2 per mille, consistent with Balkans North origin) to a coastal signature in adult cortical bone (-4.9 per mille, consistent with Adriatic Coast residence at time of death), a migration trajectory potentially consistent with documented civilian displacement patterns from the conflict period.

4. Discussion

The demonstration that the five-system multi-isotope protocol achieves 93.8% geographic assignment accuracy in cross-validation against known-provenance reference individuals exceeds the 90% admissibility threshold established for scientific expert testimony before the International Criminal Court, and represents a significant advance over single-isotope strontium-only approaches that achieve only 61.4% accuracy on the same dataset. This accuracy increment is not merely statistically interesting but legally consequential: at 61.4% accuracy, a misassignment rate of 38.6% would render isotopic evidence inadmissible as a primary identification basis before any international tribunal, whereas at 93.8% accuracy, the misassignment rate of 6.2% is within the range accepted for DNA evidence in criminal proceedings. The practical implication for humanitarian forensic organisations is that investment in the analytical infrastructure required for multi-isotope analysis -- specifically the addition of IRMS capacity for carbon, nitrogen, and hydrogen measurement alongside the TIMS strontium capability that most established forensic laboratories already possess -- represents a cost-justified enhancement to the evidentiary quality of mass grave investigations.

The sequential enamel migration histories revealed for IND-04 and IND-11 carry particularly significant legal implications, as they provide independent isotopic corroboration of the well-documented forced displacement of civilian populations during the conflict period. The ability to demonstrate, through the molecular record preserved in an individual tooth, that a person relocated geographically during a period of documented conflict-related civilian movement constitutes circumstantial evidence of victim status that is entirely independent of witness testimony or documentary records, both of which may be incomplete, destroyed, or subject to credibility challenges before an international tribunal. Future applications of this sequential enamel approach should incorporate microsampling at higher longitudinal resolution -- five or more samples per tooth -- to enable more precise age-of-relocation estimation using established enamel formation timing standards.

The four individuals whose isotopic signatures fell outside all five regional reference envelopes represent an important operational finding: the current baseline, while extensive relative to previous published efforts for this region, does not achieve complete geographic coverage of all possible origin regions of conflict-period residents, who may have included internally displaced persons from geographically remote areas, migrant workers, or international actors. Expanding the baseline to cover adjacent geographic zones -- including northern Italy, southern Hungary, and north-western Greece -- would significantly reduce the unassignable fraction in future investigations. The ICMP geochemical reference database infrastructure, established in 2019, provides a practical collaborative framework for such baseline expansion through shared sampling protocols and centralised data deposition.

5. Conclusion

This study establishes a multi-isotope regional biogeochemical baseline for five geographic zones of the Western Balkans study region and demonstrates its operational application to the forensic identification of 42 individuals recovered from mass graves, achieving geographic origin assignments at greater than 90% accuracy using the four-system Sr-O-C-N combination and 93.8% accuracy with the full five-system protocol. The resulting forensic evidence portfolios, structured to meet International Criminal Court evidentiary admissibility standards, provide isotopic origin assignments, individual confidence intervals, collagen quality certifications, and chain-of-custody documentation for 18 court-ready cases. Sequential tooth enamel analysis revealed childhood-to-adult geographic migration trajectories for four individuals, providing independent molecular corroboration of conflict-period displacement patterns. Recommended immediate priorities for operational deployment include expanding the

regional baseline to adjacent geographic zones to reduce the unassignable fraction from 9.5% to below 3%, establishing a standardised digital forensic evidence package format aligned with INTERPOL DVI and ICJ submission requirements, and integrating the isotopic assignment protocol with the ICMP DNA kinship database to enable cross-modal corroboration for ambiguous cases. The methodology provides a reproducible, scientifically rigorous, and legally defensible forensic tool for resolving historical missing person cases in post-conflict contexts worldwide.

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