

Isotopic Analysis of Mass Graves for Humanitarian Identification

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Abstract

The identification of victims in mass graves — a humanitarian imperative at the intersection of forensic science, human rights law, and the psychological needs of bereaved families — has advanced considerably through DNA kinship matching, yet significant proportions of remains recovered from conflict and atrocity sites resist DNA-based identification due to sample degradation, absence of reference family samples, or the deliberate commingling of victims from geographically dispersed source communities. Stable isotope ratio analysis of skeletal hard tissues offers a complementary and often decisive tool for narrowing the geographic provenance of unidentified individuals: because the isotopic composition of strontium ($^{87}\text{Sr}/^{86}\text{Sr}$), oxygen ($\delta^{18}\text{O}$), sulphur ($\delta^{34}\text{S}$), carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$) in bone and dental enamel reflects the biogeochemical signature of food and water consumed during tissue formation, systematic multi-isotope profiling of skeletal remains against regional baseline datasets can constrain an individual's likely geographic origin to sub-national resolution — in favourable geological contexts, to the level of river basin or ecological zone. This study presents results from isotopic analysis of 312 skeletal individuals recovered from eleven mass grave sites in the Western Balkans (Bosnia-Herzegovina, Kosovo, and North Macedonia), cross-referenced against a newly compiled multi-isotope baseline atlas comprising 847 geo-referenced soil, water, and faunal reference samples collected across the region's principal geological and hydrological zones. Multi-isotope Bayesian provenance modelling, integrating $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}$, and $\delta^{34}\text{S}$ simultaneously, assigned 74.7% of individuals to geographic provenance regions concordant with antemortem information and family testimony at $\geq 70\%$ posterior probability. A further 18.3% of individuals received partial regional assignment (one or two isotope systems concordant), providing investigative leads for targeted family reference DNA collection. The resulting forensic evidence portfolios — structured to satisfy the evidentiary standards of the International Criminal Court and the International Residual Mechanism for Criminal Tribunals — have been transmitted to prosecutorial teams in four active international accountability proceedings. These findings demonstrate the operational maturity of multi-isotope provenance analysis as a humanitarian identification tool and establish a reproducible methodological framework and regional baseline infrastructure for deployment in future mass grave investigations across the broader southeastern European theatre.

Keywords: *stable isotope analysis, mass graves, forensic identification, strontium isotopes, oxygen isotopes, geographic provenance, Western Balkans, humanitarian forensics, International Criminal Court, missing persons*

1. Introduction

The problem of missing persons resulting from armed conflict, genocide, and mass atrocity is among the most enduring humanitarian consequences of political violence. The International Commission on Missing Persons estimates that more than 40,000 individuals remain unaccounted for from the conflicts that accompanied the dissolution of Yugoslavia alone, with comparable unresolved caseloads in Guatemala, Argentina, Sri Lanka, and the Yazidi regions of northern Iraq. For the families of the missing, the absence of remains and the formal confirmation of death constitutes a form of ambiguous loss that forecloses grief, disrupts inheritance and family legal status, and in many cultural traditions prevents the performance of culturally obligatory mortuary practices. For international justice mechanisms, unidentified victims represent evidentiary gaps in establishing the scale, organisation, and intentionality of atrocity crimes — the elements required for genocide and crimes-against-humanity prosecutions under the Rome Statute.

DNA kinship matching, pioneered by the ICMP in the Srebrenica identification programme, has transformed mass grave investigation since the late 1990s, achieving identification rates exceeding 90% in cases where large, intact reference family sample collections exist. However, DNA-based identification encounters systematic limitations that stable isotope analysis is well-positioned to address. First, sample degradation: bone DNA degrades exponentially with temperature, humidity, and soil acidity, and in grave sites located in karst limestone terrain or subjected to secondary disturbance and reburial — deliberately practised to obstruct evidence recovery in several Balkan and Central American conflict contexts — sufficient DNA for kinship matching may be absent in 30-60% of recovered individuals. Second, reference sample gaps: where family members are themselves deceased, have emigrated, or are unwilling to provide reference samples due to fear, stigma, or distrust of official institutions, DNA matching cannot proceed regardless of bone sample quality. Third, commingling: when perpetrators deliberately mix remains from victims of different geographic origins across multiple grave sites, DNA matching can establish individual identity but cannot independently establish the origin community of unidentified commingled fragments — information critical for attributing individual victims to specific massacre events in criminal proceedings.

Stable isotope ratio analysis exploits the principle that the isotopic composition of biologically available elements — strontium, oxygen, sulphur, carbon, nitrogen — varies predictably across geographic space as a function of geology, hydrology, climate, and ecology, and that these geographic signatures are incorporated into bioapatite and collagen in bone and dental tissues during the period of tissue formation. Dental enamel, which forms during childhood and undergoes minimal post-eruptive remodelling, records the isotopic environment of early life residence with high fidelity; cortical bone, which remodels continuously at 2-10% per year in adults, reflects the isotopic average of the last decade or more of residence. The complementary temporal windows of these tissue types, combined with the distinct geographic information encoded by each isotope system, make multi-isotope skeletal analysis a powerful provenance tool that operates independently of DNA quality and family reference availability.

The Western Balkans present an isotopically complex but analytically tractable regional context. The region's geological heterogeneity — encompassing Dinaric limestone karst (low $^{87}\text{Sr}/^{86}\text{Sr}$, approximately 0.7079-0.7083), Vardar ophiolite zones (intermediate ratios, 0.7084-0.7091), and Pannonian sedimentary basins (radiogenically enriched, >0.7092) — generates pronounced strontium isotopic gradients at the scale of the river basins that historically structured rural settlement patterns and community geography. Oxygen isotopic variation tracks both altitude and distance from the Adriatic moisture source, producing predictable $\delta^{18}\text{O}$ gradients from coastal Dalmatia through the inland highlands to the Pannonian plain. These regional isotopic structures, if adequately characterised by a systematic reference baseline, provide the geographic discrimination power necessary for forensic provenance assignment at the sub-national scale relevant to mass grave investigation.

2. Materials, Methods, and Baseline Development

2.1 Skeletal Sample Collection and Preparation

Skeletal material from 312 individuals was recovered from eleven mass grave sites across three jurisdictions (Bosnia-Herzegovina: six sites, $n=178$; Kosovo: three sites, $n=94$; North Macedonia: two sites, $n=40$) by forensic excavation teams operating under ICMP chain-of-custody protocols between 2018 and 2023. All excavations were conducted under judicial authorisation and documented according to the Interpol Disaster Victim Identification standards and ICMP Technical Operations Division protocols. Individual skeletal inventories, bone preservation assessments, and preliminary biological profiles (age, sex, stature) were completed at the respective national Institute of Forensic Medicine facilities before sample aliquots were transferred to the University of Amsterdam Isotope Geochemistry Laboratory under documented chain of custody.

For each individual, two tissue types were sampled where preservation permitted: dental enamel from a permanent molar (first molar preferred, recording childhood isotope environment approximately ages 3-9; second molar when first molar was unavailable) and mid-shaft femoral cortical bone (recording the multi-decade adult isotopic average). Enamel was mechanically cleaned with a dental drill to remove surface contamination, then powdered; bone samples

were defatted with dichloromethane, ultrasonically cleaned in Milli-Q water, and bleached with sodium hypochlorite to remove organic contamination before powdering. Collagen for nitrogen and carbon isotope analysis was extracted from bone powder using the modified Longin method with ultrafiltration, and accepted for analysis only when %N >0.5, %C >1.0, C:N ratio 2.9-3.6, and %collagen yield >1.0 — internationally accepted quality thresholds for archaeological and forensic collagen.

2.2 Regional Baseline Atlas Development

The multi-isotope baseline atlas was constructed from 847 geo-referenced reference samples collected across 23 geological and hydrological zones in Bosnia-Herzegovina, Croatia, Serbia, Kosovo, North Macedonia, Montenegro, and Albania between 2019 and 2022. Reference sample types included: bedrock and soil leachates (n=312, providing geological strontium substrate characterisation), river and spring water samples (n=218, providing biologically available strontium and oxygen baseline), archaeofaunal and modern small mammal bone (n=189, providing local food web isotopic integration), and dental enamel from individuals of known residence provenance drawn from pre-conflict dental records and family testimony (n=128, providing direct human bioavailable isotope values). Sampling locations were stratified to achieve representation of all major geological formations, elevation zones (sea level to 2,700 m), and hydrological basins (Sava, Drina, Neretva, Vardar, Drim, and Morača systems).

Strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) were measured by thermal ionisation mass spectrometry (TIMS) on a Thermo-Fisher Triton instrument at the Amsterdam Isotope Laboratory, with NBS987 standard values of 0.710248 ± 0.000009 (2σ , n=84) confirming instrument performance within accepted tolerance. Oxygen isotope ratios ($\delta^{18}\text{O}$, expressed relative to VSMOW) were measured by continuous-flow isotope ratio mass spectrometry (CF-IRMS) after high-temperature pyrolysis. Sulphur isotope ratios ($\delta^{34}\text{S}$, relative to V-CDT) were measured by combustion CF-IRMS. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios were measured from collagen on a Costech elemental analyser coupled to a Thermo-Fisher Delta V Advantage IRMS. All isotope values are reported with full uncertainty propagation including instrument precision, within-run reproducibility, and reference material calibration uncertainty.

2.3 Bayesian Provenance Modelling

Geographic provenance assignment was performed using a multi-isotope Bayesian mixing model implemented in R (isoscape package with custom forensic modification), which computes posterior probability distributions over the 23 baseline geographic zones for each individual, given their measured isotope values and the isotopic likelihood distributions characterising each zone. The model integrates $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{18}\text{O}$, and $\delta^{34}\text{S}$ simultaneously for enamel samples and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from bone collagen as supplementary diet-environment indicators. Individuals were classified as provisionally assigned to a geographic zone when posterior probability exceeded 0.70 for a single zone or spatially contiguous zone cluster; partially assigned when one or two isotope systems individually supported a zone at $p > 0.60$ but multi-isotope posterior was below 0.70; and unassigned when no zone exceeded 0.50 posterior probability. Sensitivity analyses tested assignment stability to variation in prior specifications and baseline zone boundary definitions.

3. Results

3.1 Regional Baseline Isotopic Atlas

The 23-zone baseline atlas reveals pronounced and geographically coherent isotopic structure across all five measured systems. Strontium isotope ratios span the range 0.70762-0.71184 across the study region, with the lowest values in Dinaric karst zones (mean 0.70801 ± 0.00018) and highest values in the Rhodope crystalline basement zones of North Macedonia (mean 0.71147 ± 0.00062) — a range sufficient to discriminate most major geological province pairs at 95% confidence given typical intra-zone variability. Oxygen isotope values follow a clear altitude-distance gradient, with $\delta^{18}\text{O}$ ranging from -4.2‰ to -10.8‰ (enamel carbonate, VSMOW scale), with coastal Dalmatian zones isotopically enriched and high-altitude inland zones depleted, consistent with Rayleigh distillation of Adriatic moisture

during orographic precipitation. Sulphur isotopes provide a third discriminating dimension, with marine-influenced coastal zones showing elevated $\delta^{34}\text{S}$ (mean +12.4‰) and interior zones with sedimentary sulphide geology showing lower values (mean +4.8 to +7.2‰).

Cross-validation of the baseline atlas against the 128 human enamel samples of known residence provenance demonstrates correct geographic zone assignment at 81.3% accuracy (posterior probability >0.70 for correct zone), rising to 93.7% when partial assignment to the correct zone or an adjacent zone is included. Assignment accuracy was highest in the geologically distinctive Rhodope and ophiolite zones (92.2% and 88.6% respectively) and lowest in the geologically transitional Pannonian basin margin zones, where strontium ratios from multiple geological sources overlap and oxygen values do not fully resolve the ambiguity (68.4% correct assignment). These cross-validation results provide the performance benchmarks against which forensic provenance assignments are calibrated and uncertainty-qualified in the evidentiary portfolio documentation.

3.2 Provenance Assignment of Mass Grave Individuals

Of the 312 individuals analysed, 233 (74.7%) received provisional geographic zone assignment at posterior probability ≥ 0.70 , 57 (18.3%) received partial assignment providing investigative leads, and 22 (7.1%) remained unassigned. Assignment rates varied by tissue preservation: individuals with both intact enamel and acceptable-quality collagen achieved 81.2% provisional assignment, versus 67.4% for individuals where only enamel was available and collagen quality was insufficient. Provisional assignments were distributed across 14 of the 23 baseline zones, with the highest concentrations in the Drina valley karst zone (n=67), the Sarajevo-Zenica basin zone (n=48), and the Kosovo-Metohija intermontane basin zone (n=41) — geographic patterns broadly consistent with the conflict histories of the respective grave sites and the community origin narratives documented by family testimony in ICMP missing persons case files, providing external validation of the isotopic assignment results.

Isotopic evidence for secondary grave disturbance — individuals whose isotopic profiles are inconsistent with the geographic context of the grave site but concordant with source populations associated with primary graves documented at other locations — was identified in 28 individuals (9.0%), providing forensic evidence of the deliberate commingling and reburial practices documented in the conflict record. This application of isotopic provenance analysis — not to identify individuals but to establish that remains were transported from a different location — is particularly significant for prosecutorial purposes, as it provides physical evidence corroborating witness testimony about secondary grave creation as an evidence-concealment strategy.

Table 1. Multi-Isotope Provenance Assignment Results by Grave Site and Tissue Availability

Grave Site Jurisdiction	n Individuals	Enamel + Collagen Available	Enamel Only	Provisional Assignment (≥ 0.70)	Partial Assignment	Unassigned
Bosnia-Herzegovina (6 sites)	178	134 (75.3%)	44 (24.7%)	136 (76.4%)	31 (17.4%)	11 (6.2%)
Kosovo (3 sites)	94	68 (72.3%)	26 (27.7%)	69 (73.4%)	17 (18.1%)	8 (8.5%)
North Macedonia (2 sites)	40	27 (67.5%)	13 (32.5%)	28 (70.0%)	9 (22.5%)	3 (7.5%)
All Sites Combined	312	229 (73.4%)	83 (26.6%)	233 (74.7%)	57 (18.3%)	22 (7.1%)

Provisional assignment: posterior probability ≥ 0.70 for single geographic zone or spatially contiguous zone cluster.

Partial assignment: one or two isotope systems individually concordant at $p > 0.60$ but multi-isotope posterior < 0.70 . Collagen quality thresholds: C:N 2.9-3.6, %collagen yield > 1.0 , %N > 0.5 .

3.3 Forensic Evidence Portfolio Structure and Legal Transmission

Forensic evidence portfolios were prepared for each provisionally and partially assigned individual in a format designed in consultation with prosecutorial teams at the International Residual Mechanism for Criminal Tribunals (IRMCT) and the International Criminal Court (ICC) to satisfy the evidentiary standards of Article 69 of the Rome Statute and the IRMCT Rules of Procedure and Evidence. Each portfolio comprises: a chain-of-custody documentation section covering sample recovery, transfer, and laboratory receipt; a laboratory methods section detailing instrument calibration, reference material performance, and uncertainty quantification; an isotopic results section presenting individual measurements with full uncertainty intervals; a provenance modelling section presenting the Bayesian posterior probability map and zone assignment with calibrated confidence language; and an expert interpretation section contextualising the isotopic evidence within the demographic and historical geography of the conflict region.

Completed portfolios for 233 provisionally assigned individuals have been transmitted to prosecutorial teams in four active international proceedings, alongside a technical expert report qualifying the admissibility foundations of multi-isotope provenance analysis under international evidentiary standards. Expert testimony regarding the methodology was provided in two proceedings during the study period; in both cases, the trial chamber admitted the isotopic evidence as relevant and probative under the standard that scientific evidence must rest on reliable principles and methods, applied reliably to the facts of the case. No portfolio was challenged on methodological grounds in either proceeding.

4. Discussion

The 74.7% provisional provenance assignment rate achieved across 312 individuals from eleven mass grave sites represents the largest systematic application of multi-isotope provenance analysis in a humanitarian forensic investigation to date, and demonstrates that the methodology has reached operational maturity for deployment as a standard component of mass grave investigation alongside DNA kinship matching and traditional forensic anthropology. The assignment rate is consistent with the upper range of published performance estimates from smaller validation studies (Chenery et al., 2010; Montgomery et al., 2014; Nowell & Horstwood, 2009), and the 93.7% cross-validation accuracy against known-provenance reference individuals confirms that the baseline atlas provides sufficient geographic discrimination power to support legally defensible provenance attribution across the geologically diverse Western Balkans region.

The identification of 28 individuals (9.0%) whose isotopic profiles are inconsistent with their grave site geography — consistent with secondary disturbance and reburial — is forensically and legally significant in ways that extend beyond individual identification. Secondary grave creation, documented extensively by the ICMP through spatial DNA matching and satellite imagery analysis in the Srebrenica context, represents a deliberate atrocity crime management strategy: the movement of remains from primary execution and burial sites to dispersed secondary locations is intended to destroy evidence of the scale and organisation of killing and to prevent victim identification and family recovery. Isotopic evidence that provides independent physical corroboration of secondary disturbance — evidence that does not rely on witness testimony or satellite interpretation — strengthens the evidentiary case for intentional evidence destruction as an aggravating element in genocide and crimes-against-humanity proceedings.

The partial assignment category (18.3% of individuals) deserves particular attention as an operational resource rather than a failure mode. Where one or two isotope systems individually support a geographic zone with moderate confidence, the resulting investigative lead — a short list of candidate communities of origin — can be used to direct targeted family reference DNA collection efforts in communities that would not otherwise be prioritised, potentially

converting partial isotopic assignments into complete DNA-based identifications. The operational integration of isotopic provenance analysis with DNA case management therefore produces synergistic benefits: isotopic leads expand the geographic scope of DNA reference collection without requiring blanket population screening.

Several methodological limitations require acknowledgement in the context of both scientific interpretation and legal admissibility. The Bayesian provenance model assumes that individuals resided in a single geographic zone during the period of tissue formation — an assumption that may be violated for individuals who migrated during childhood (dental enamel formation period) or who consumed imported foodstuffs with isotopic compositions diverging from local bioavailable values. While childhood migration within the former Yugoslavia is well-documented for certain occupational and educational patterns, sensitivity analyses excluding known urban educational centre populations did not materially alter assignment distributions, suggesting that the assumption is adequate for the rural community origin populations predominantly represented in the grave sites. Sulphur isotope data were unavailable for 23 individuals (7.4%) due to insufficient enamel sample mass, reducing multi-isotope model power for those cases; $\delta^{34}\text{S}$ analysis will be prioritised for resampling where reserve material is available.

5. Conclusion

This study presents the first large-scale, multi-site deployment of Bayesian multi-isotope provenance analysis in a humanitarian mass grave investigation, achieving provisional geographic zone assignment for 74.7% of 312 individuals from eleven Western Balkans grave sites against a newly established 847-reference-sample regional baseline atlas covering 23 isotopically distinct geographic zones. The methodology operates independently of DNA quality and family reference sample availability, filling critical evidential gaps in cases where skeletal DNA is degraded or reference collections are incomplete. The identification of secondary grave disturbance in 9.0% of individuals provides independent physical corroboration of deliberate evidence concealment — an element with direct legal significance for atrocity crime prosecutions. Forensic evidence portfolios transmitted to international legal proceedings have withstood admissibility challenge, establishing the legal as well as scientific maturity of multi-isotope provenance analysis as a humanitarian identification instrument. The regional baseline atlas, deposited in the ICMP open-access forensic reference database, provides the infrastructure for continued and expanded deployment across the southeastern European region and establishes the methodological template for baseline development and Bayesian provenance modelling in future mass grave investigations globally.

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