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The effects of decomposition and environment on antemortem H-Pb-Sr isotope compositions and degradation of human scalp hair: Actualistic taphonomic observations

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ABSTRACT

Multi-isotope analysis (*e.g.*, Sr–Pb–O–H–C–N) of human scalp hair is routinely used in forensic investigations of human remains to constrain the geographic origin of unidentified bodies, and to investigate antemortem mobility patterns. However, while it is known that postmortem processes can affect the preservation of, or even overprint, the biogenic isotopic signatures in hair, the speed and nature of these processes have rarely been studied. This study investigates the effects of decomposition and environment on the H–Pb–Sr isotope compositions of human hair as well as the relationship between structural hair shaft degradation and isotopic signature change over time. Human scalp hair samples from four body donations were collected at different stages throughout gross body decomposition. The willed-donated bodies were placed to decompose outdoors at the Forensic Anthropology Research Facility (FARF) at Texas State University. Hair fibers from two of the donations were examined using scanning electron microscopy (SEM) and high-resolution light microscopy (HRLM). Chemical and microbiological degradation of hair fibers occurred rapidly after placement of the body outdoors. Measurements of scalp hair isotopic composition demonstrated that H–Pb–Sr isotope ratios were altered within days after environmental exposure, presumably by deposition, leaching and/or exchange with the local bioavailable soil, and vapor. The degree of physical hair degradation and changes in H–Pb–Sr isotope composition were not correlated. We conclude that antemortem isotopic H–Pb–Sr isotope ratios are difficult to recover in hairs derived from decomposing whole bodies.
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1. Introduction

Over the past decade, the application of stable and radiogenic isotope systems has become a staple of forensic investigations (*e.g.*, anthropological case work [1,2]; wildlife forensics [3,4]; and food forensics [5]). In combination with reference databases and (predictive) reference isotope maps (isotopic landscapes or *isoscapes*), the application of multi-isotope analysis of keratin (hair, nails), bones (apatite, collagen), and dental remains (enamel, dentine) in medicolegal case work provides valuable information about the dietary preferences and geolocation at various times during life of unidentified human remains. Dental enamel provides insights into residential stability or mobility and diet during childhood, while bones exhibit average isotopic signatures representative of the food consumed over a period lasting from weeks (foetal bones) to decades (adult femur diaphyses) prior to death. The period of life that is represented by the bone sample depends on skeletal element and part (diaphysis/epiphysis), type of bone (cancellous/cortical), bone remodeling rate, sex, age at death, general health and mechanical stress applied to the bone [6]. Scalp or pubic hairs provide sequential information about geographical movement and dietary changes or preferences of the

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deceased in the weeks to years prior to death, and are thus particularly valuable for narrowing the search of missing persons for identification purposes.

While it is known that postmortem processes can affect the preservation of, or even overprint, the antemortem biogenic isotopic signatures in hair, the study of the effects of environmental exposure and biodegradation on the physical structure of the scalp hair shafts and the isotopic integrity of the hair is still in its early stages [7–9]. As a part of a larger ongoing actualistic experimental study on human decomposition at the outdoor human decomposition facility managed by the Forensic Anthropology Center at Texas State University (FACTS) [10–12], human scalp hair samples were collected between April 2015 and August 2017 from four willed-donated human bodies from burial and open pit deposition contexts at different stages of gross body decomposition for microscopic and hydrogen (H), lead (Pb), and strontium (Sr) isotope analyses (hereafter referred to as H, Pb, and Sr respectively). The objectives of this research were to study the effects of degradation and environment on the integrity of the antemortem isotope ratios and physical properties of human hair during decomposition, and to (re-)assess the potential and limitations of isotopic analysis of human hair for provenance purposes in forensic investigations.

2. Forensic isotope analysis

Standard forensic identification methods include the documentation of intentional body modification, and DNA, fingerprint, dental and X-ray comparisons [13]. These methodologies require comparison to individuals' antemortem records. If, due to the absence of antemortem information, or the recovery of fragmented, skeletonized or badly preserved human remains, positive identification cannot be established, isotope research potentially provides useful information that aid in victim identification. Isotope forensics also requires comparative databases or maps to allow for accurate interpretations, but these are derived from predictive (computer) models or maps based on geological, climatic and dietary data [1,14,15], rather than individuals' records. In recent years, predictive models for forensic purposes for a number of isotope systems have been generated for some parts of the world, such as for Sr [16,17], Pb [18], oxygen (O) [14,19], and H [19]. In addition to information on peri-mortem geolocation derived from these elements, the stable isotope systems of carbon, nitrogen and sulfur can be used to infer information about diet, which in specific cases can indicate geolocation [20,21], malnourishment or disease [22,23]. To provide as much information as possible to aid the forensic human anthropological identification process, multiple lines of evidence, *i.e.*, multiple isotope systems, are applied (for examples see [15,24]).

The isotopic signature of hydrogen in hair keratin is mainly controlled by diet and drinking water sources (derived from local atmospheric sources) during the growing phase. Post-synthesis isotopic alterations, however, do occur due to the fact that between circa 9%–17% of structural hydrogen in hair keratin 'is subject to continuous isotopic exchanges with the environment'; *i.e.*, equilibrating with the environment in which they are stored and/or measured [19,25,26]. Consequently, interlaboratory isotopic differences exceeding 10 ‰ have been observed [25]. Nevertheless, the non-exchangeable $\delta\ ^{2}H$ values should provide valuable insights into the region of origin of unidentified human remains at different stages in life [14]. The nonexchangeable hydrogen in hair, however, may be subject to isotopic offsets after death and sampling due to the choice and time of storage (*e.g.*, freezing: [27,28]), packaging material and laboratory methodology hampering accurate interpretations and the forensic investigations.

Pb in hair keratin comes from three different sources: endogenous, semi-exogenous and exogenous [29]. Pb from the first two sources are taken up from the bloodstream through the growing shaft in the follicle and adsorbed from sebaceous and sweat gland secretions respectively [29]. Both reflect the Pb that has been incorporated in the body through diet. The exogenous source represents contaminants on the outer surface of the hair strands, and includes both anthropological inputs and geological sources; such as soil, dust, dyes, *etc.* Little research has been conducted on the separation of exogenous and endogenous Pb isotope signatures. Recent research by Gordon et al. [9], however, gave a preliminary, but strong indication that despite applying a tested, aggressive cleaning method [30], biogenic endogenous Pb isotope ratios were extremely challenging or possibly even impossible to recover.

Strontium in hair derives from two distinct pools: an endogenous source and an exogenous source (*e.g.*, [30]). The former source of Sr in hair keratin originates from the ingested fluids and food and is incorporated into the hair during the growth cycle. The exogenous contaminating source represents external factors, independent of dietary preferences, such as environmental waters (ground water, precipitation, bathing *etc.*), natural and anthropogenic aerosols (dust, fog, smoke, *etc.*) and particulate matter (*e.g.*, soil, [31] and references therein).

3. Microscopic hair analysis

The effects of taphonomy on hair fibers have been studied in archaeological and experimental contexts, providing insight into the types of damage and degradation associated with different variables [8,32–35]. The increased interest in human hair samples for identification purposes using stable isotope and trace element analysis highlights the importance of understanding the degradation of human hair fibers over time. This has been little investigated. Experimental work on human hair degradation provides an important framework for documenting and evaluating histological markers of human hair degradation [34,36]. Wilson and colleagues [34,36] microscopically documented changes in morphological features of human hair fibers using high resolution light microscopy (HRLM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Based on the changes and damages in morphological features they developed a histological scoring method to assess the degree of degradation of a hair sample, which was used as a framework in the present study.

4. Environmental setting

The willed-donated bodies were placed outdoors at the Forensic Anthropology Research Facility (FARF) in central Texas. FARF's terrain consists of shallow clay-rich soil overlying deposits of limestone with grassland punctuated by woodlands of primarily oak and cedar trees [37]. The region has a semi-humid climate with hot summers and moderate winters. The mean average temperature is 19.4 °C (68 °F), but temperatures of 32 °C (90 °F) or greater occur in every month but December and January [38]. On average, the region experiences approximately 113 days per year with temperatures that exceed 32 °C. Freezing is uncommon, occurring on average 36 days per year, but low temperatures reaching the freezing point have been reported in all but five months (May through September: [38]).

The predominant vegetation consists of scrub bushes, ashe juniper (*Juniperus ashei*), and Texas live oak trees (*Quercus fusiformis*). The soils in the Texas Hill Country are primarily shallow, stony and overlay Cretaceous limestone. At FARF the two types of soils are 1) Rumple-Comfort Association, Ungulating

(RUD) and 2) Comfort-Rock Outcrop Complex, Undulating (CrD). Both are characterized by relatively shallow, rocky, clay top soils. Both soil types are also relatively alkaline with high carbonate content and relatively low organic matter. Because of the high clay and rock content, the soils at FARF have relatively low permeability to air and water movement [39–41]. The available soil Sr concentration data show intermediate values (170–300 ppb: [9]).

Annual precipitation is 81 cm, but the region experiences frequent droughts and flash flooding. Snowfall does not occur most winters. The concentration of Sr in precipitation at the Texas facility is low (2–8 ppb), while the "hard" ground water exhibits Sr concentrations ranging between 8000–1000 ppb (8–10 ppm: [9]).

5. Material

5.1. Forensic Anthropology Center at Texas State University – body donation program

FACTS receives whole body donations for scientific research under the Texas revised Uniform Anatomical Gift Act (National Conference of Commissioners on Uniform State Laws 2009). Body donations are exclusively acquired by Texas State University through the expressed and documented willing of the donors and/or their legal next of kin. Body donations are made directly to FACTS, and donors and/or their next of kin are aware that donations are used for taphonomic studies. Demographic, health, and other information are provided through a questionnaire completed by the donor or legal next of kin. The data are securely curated by FACTS. The body donation program complies with all legal and ethical standards associated with the use of human remains for scientific research.

5.2. Placement of willed-donated bodies

The willed-donated bodies of four females, aged between 61 and 91 years old with European ancestry type hair, were placed unclothed to decompose outside at FARF in central Texas between April 2015 and October 2015. Time of death ranged between 2–10 days prior to placement. Body donations were stored in coolers at 4 °C prior to placement. Each body was in a fresh stage of decomposition upon placement, following Megyesi et al. [42]. Two body donations (1 and 4) were placed in hand dug oval shaped pits, measuring approximately 65 by 95 cm wide and 70 cm deep, which remained open throughout the study, and were observed directly while decomposition of soft tissue, skeletonization and joint disarticulation occurred. Two body donations (2 and 3) were placed in shallow oval shaped pits of similar dimensions and buried with the soil from the pits, simulating shallow clandestine burial contexts. All donations were placed well above the ground water level.

5.3. Hair sample collection

Scalp hair samples of buried bodies (donations 2 and 3) were taken in 2015 during intake of the bodies in fresh state ("A" samples, prior to placement outside) and in 2017 during retrieval of the remains in skeletonized state ("C" samples). Hair samples of bodies left to decompose in an open pit (donations 1 and 4) were taken in 2015 during intake of the bodies in fresh state ("A") and approximately one week later when the body was in a state of active decomposition ("B"). Final "C" samples for these two open pit burials were taken when the head was in skeletonized state at the earliest, and during retrieval of the remains in skeletonized state at latest. All biological and hair specimen data are provided in Table 1. Information on hair color and condition can be found in the Supplementary data.

Circa 100 hairs per sample were collected using tweezers. Hairs were extracted at the root when possible. Next, if practicable, all strands were aligned along the direction of growth and subsequently wrapped in aluminum foil and packaged in Ziploc storage bags. They were transferred to a lockable freezer at −20 °C and shipped overnight on dry ice to the Vrije Universiteit Amsterdam. Upon arrival, the samples were immediately transferred to a lockable freezer at −20 °C.

6. Methods

6.1. Documenting decomposition and environmental conditions

Data on body decomposition and environmental conditions were collected throughout the study (see Supplementary data). Gross decomposition was observed and documented daily using photography and a written form. The temporal sequence of the postmortem interval was recorded using accumulated degree-days (ADD) [42]. Decomposition was quantified using the total body score (TBS) method following previously published guidelines [42]. Weather was recorded at three weather stations: one located approximately one mile from the facility and accessible through the MesoWest Surface Weather Service, and two HOBO Micro Station data loggers located on the facility. Data were recorded at 30-min intervals at all stations and include ambient air temperature, humidity, wind speed, wind direction, solar radiation, precipitation, and other climatic variables.

6.2. Cleaning of scalp hair

Prior to sample preparation, decontamination was performed. Plastic tweezers were used to remove large parts of any adherent soil. To remove the remaining adherent soil and dust particulates, the hairs were placed between two layers of brass netting and blasted with high pressure N_2 gas for circa 30 s. Next, the biosamples were washed successively 6 times in Milli-Q water (Millipore, US) and 5 times in a mixture of methanol and chloroform (1:2 v/v; purities >99%) using a vacuum filter apparatus. Each washing step took circa 30 s. The "A" samples were then dried in a fume hood at room temperature for 48 h. To remove the last of the adherent soil, the "B" and "C" samples were sonicated twice for 20 s in Milli-Q water and rinsed in Milli-Q water, at which point no further contamination could be visually identified. The samples were dried following the same protocol as the "A" samples.

6.3. Microscopy

6.3.1. Scanning Electron Microscopy (SEM)

SEM was used to assess degradation of the external structure of the hair fibers. From each sample 10 hairs were randomly selected. A 1 cm section of the shaft was taken from the middle of each individual hair. The sections were coated with a thin layer of carbon to increase secondary-electron yield, and then examined and documented using secondary electron imaging (SEI) with a JCM-6000 JEOL Scanning Electron Microscope (Tokyo, Japan) under high vacuum with an electron beam energy at 15 kV.

6.3.2. High Resolution Light Microscopy (HRLM)

HRLM was used to assess degradation of the internal structure of cross-sectioned hair fibers. Approximately 20–30 hairs per donation were embedded in paraffin. Following Thavarajah and colleagues [43], the hairs were fixed in 4% formaldehyde overnight, to protect them against external damage, to increase their mechanical strength, and to preserve their morphology. Next, the samples were incubated in warm paraffin overnight, and

Table 1
Biological and hair specimen data. All willed-donated bodies were biological females, aged between 61 and 91 years at death. *T-1 = day before interment/placement; T0 = day of interment/placement.

Donation	Hair length (cm)	Time as resident of Texas immediately prior to death	Time since death upon placement in days	Deposition context	Sample ID	Collection date (dd-mm-yy)	Time of sample collection in days*	Remark
1	6	4 years	10	Open pit	H1A	28-04-2015	−1	Intake sample prior to placement
					H1B	04-05-2015	6	Second sample during active decomposition
					H1C	03-12-2015	219	Final sample upon retrieval of skeletal remains
2	3–4	7 months	2	Burial	H2A	07-05-2015	0	Intake sample prior to placement
					H2C	–	–	No hair was found preserved upon excavation of the remains
3	45	50 years	3	Burial	H3A	24-06-2015	0	Intake sample prior to placement. Hair was dyed, the first 10 cm showed the natural hair color (gray-brown)
					H3C	21-08-2017	790	Final sample upon retrieval of skeletal remains
4	10–15	Entire life	10	Open pit	H4A	19-10-2015	−1	Intake sample prior to placement
					H4B	26-10-2015	7	Second sample during active decomposition
					H4C	15-11-2015	28	Final sample during skeletonization of the head (not of the body)

allowed to solidify at room temperature the next day. Using a microtome, thin sections of 5 μm thickness were sliced from the paraffin blocks.

These thin sections were placed on a TOMO Adhesion Microscope Slide (Matsunami, Osaka, Japan) on top of a drop of 0.02% Bovine Serum Albumin (Sigma-Aldrich, Darmstadt, Germany). The slides were heated to 65 °C for 1 h to attach the small hair sections to the glass slides. The slides were stained with a 0.01% Toluidine Blue (TB) solution (Sigma–Aldrich, Darmstadt, Germany), an acidophilic metachromatic dye that stains acidic tissue components such as sulfates, carboxylates, and phosphate radicals, as well as nucleic acids (see supplementary data 2 for protocol description). The microscope slides with stained and thin sectioned hair samples were examined with a Leica DMLB light microscope (Wetzlar, Germany).

6.3.3. Assessment of hair degradation

Morphological features of degradative change in the hair samples were documented according to the scoring system developed by Wilson et al. [36], which uses a ranked histology to assess the condition of the external and internal structure of hair fibers.

6.4. Isotope analysis

6.4.1. H isotope analysis

The δ^2H isotope ratios were analyzed using a Delta XP plus isotope ratio mass spectrometer combined with a TC/EA elemental analyzer and Conflo III. Approximately 0.17 mg of chopped cleaned hair samples were weighed into 4.5 mm Ag capsules that were introduced into the IRMS via a zero-blank auto-sampler. Samples were run in triplicate. The stable hydrogen and oxygen isotope-delta values of hair samples are reported relative to the VSMOW-SLAP scale; on these scales the $\delta^2H_{VSMOW\text{-}SLAP}$ values of USGS42 and USGS43 used for calibration are −72.9 ‰ and −44.4 ‰ respectively [45,46]. Analytical precision for hair analysis, calculated from the measured hydrogen isotope ratios of chopped hair (IDIS) used as an internal secondary reference material was ±1.3 ‰ for H (n = 15). Samples and standards were equilibrated at ambient temperature in the laboratory for 5 days before measurement.

6.4.2. Pb and Sr isotope analyses

Between 3 and 8 mg of cleaned hair sample was selected and transferred to a class 100 clean laboratory at the Vrije Universiteit Amsterdam. The hairs were placed in acid-cleaned 7 mL Teflon beakers and digested in a mixture of 14 M HNO_3, concentrated HF, and 6–7 M HCl. The sample was then evaporated to dryness and the residue was taken up in 200 μL 0.7 M HBr. Acid-cleaned polyethylene pipette-tip columns were loaded with 100 μL of Bio-Rad® AG 1-X8 200–400 mesh resin and cleaned with 1 mL of 1 M HF, 6.5 M HCl, and milli-Q water before conditioning with 400 μL 0.7 M HBr. The matrix containing the Sr was washed out using 2 mL 0.7 HBr and 200 μL 3 M HCl. The elution of the Pb fraction was accomplished using 800 μL 6.5 M HCl. A 1% aliquot of the Pb fraction was taken for concentration measurements.

The Sr fraction was dried down, and subsequently dissolved in 500 μL 3 M HNO_3. Pipette columns were filled with Sr-Resin (EIChroM) and cleaned three times alternating 1.5 mL 3 M HNO_3 and Milli-Q water, before conditioning with 500 μL 3 M HNO_3. The samples were washed with 1.8 mL HNO_3 and the Sr fraction was collected using 800 μL Milli-Q water.

The 1% Pb aliquot and the Pb blanks were spiked with ^{208}Pb spike and the Sr blanks and the "A" and "C" samples were spiked with ^{84}Sr to monitor the change in Sr concentration through time. The Pb and Sr fractions were dried down, and nitrated twice with circa 150 μL 14 M HNO_3 to remove all organic fractions. The acids used during the chemistry procedures were purified by sub-boiling distillation in Teflon from initial pro-analysis grade acids (Merck).

A double spike technique in combination with the use of a $10^{13}\Omega$ resistor for the collection of ^{204}Pb was applied to produce

highly accurate and precise data for low Pb concentrated samples. A detailed description of the Pb loading procedures and the double spike technique is provided elsewhere [44]. Samples and NIST SRM981 (NBS981) standards were measured for isotope composition using a ThermoFinnigan Triton Plus thermal ionization mass spectrometer (TIMS) housed in the Department of Geology and Geochemistry at the Vrije Universiteit Amsterdam (see [44] for analytical details). The VU long-term NIST SRM981 (75 ng) gave $^{206}Pb/^{204}Pb = 16.9409 \pm 0.0009$, $^{207}Pb/^{204}Pb = 15.4983 \pm 0.0009$, and $^{208}Pb/^{204}Pb = 36.7213 \pm 0.0025$ ($\pm$ 2SD, N = 28: [44]). The total procedural blank was 25 pg. This corresponds to a 2.2%–5.0% blank contribution to the "A" samples, and a 0.3%–0.4% blank contribution to the "C" samples. Nevertheless, a blank correction was found to have no significant effect (maximum 0.02 difference in $^{208-206}Pb/^{204}Pb$).

A detailed description of the Sr sample loading procedures is given elsewhere [20]. The Sr isotope compositions were measured by TIMS. The ratios were determined using a static routine and were corrected for mass-fractionation correction. The long-term average of NBS987 during the period of research was 0.710249 ± 0.000022 (N = 62). The measurements were all normalized to 0.710240 for NBS987. The total procedural blank contained a negligible amount (<15 pg Sr).

7. Results

Upon retrieval of the skeletal remains of donation 2, 27.3 months after burial, no hair samples were retrieved. Hence, only the intake samples, "A", are included in this study.

7.1. Microscopy

Two hair samples each from donations 1 (H1A and H1C) and 4 (H4A and H4C) were examined with SEM and HRLM. Both donations were placed to decompose in shallow pits that remained open throughout the experiment. The "A" samples represent the baseline samples (upon intake of the body) to which the "C" samples (collected during skeletonized state of the entire remains or, in the case of willed-donated body 4 the skeletonized state of the skull) were compared.

Scanning electron microscopy was used to identify loss and/or damage to the external hair surface. The "A" and "C" hair samples from donation 1 showed a large difference in degree of degradation (Fig. 1). H1A showed lifting of cuticle margins and loss of and/or minor damage to the hair cuticle, which could be related to antemortem environmental factors such as weather-damage [47]. In contrast to H1A, H1C showed extensive lifting of cuticle margins, severe loss of and/or damage to the cuticle, as well as loss of cortex and/or medulla inside the hair and extensive erosive lesions in the hair shafts as a result of fungal tunneling [36].

The "A" and "C" hair samples from donation 4 showed less difference in degree of degradation (Fig. 1). H4A showed only slight lifting of cuticle margins; however, H4C showed more severe lifting of cuticle margins and loss of and/or damage to the cuticle. In contrast to sample H1C from donation 1, no loss or damage to the cortex and medulla were observed in sample H4C, nor any evidence of fungal tunneling [36].

High-resolution light microscopy allowed examination of degradation of the interior of the hair fibers. The results of the HRLM analysis are largely consistent with the findings of the SEM analysis. Analysis of cross-sectioned hairs revealed several types of hair degradation in both "A" and "C" samples from donation 1 (Fig. 2). The H1A intake sample did not exhibit a great degree of damage; in contrast, several types of degradation were found in the H1C sample. H1A exhibited limited loss of the cortex and medulla inside the hair fibers; no fungal holes were present. H1C exhibited extensive damage to, and loss of, the cortex and medulla, as well as

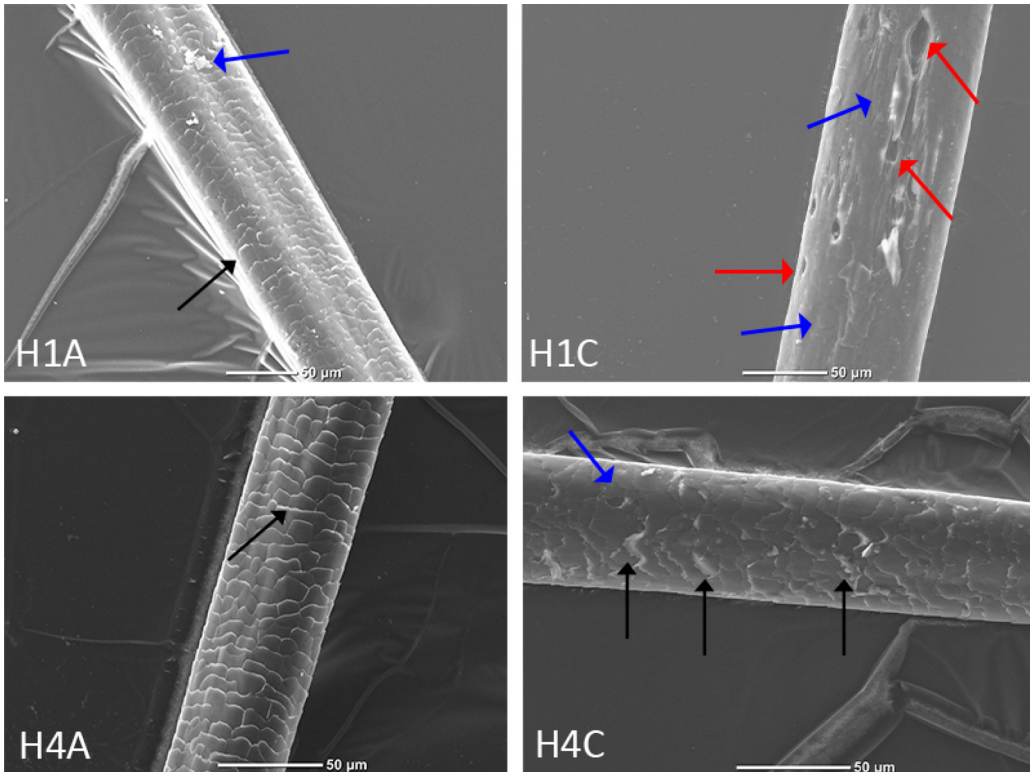


Fig. 1. SEM images of hairs from Donations 1 and 4. The difference in time between collection of samples A and C is 219 and 28 days, respectively. Red arrows indicate fungal holes, black arrows indicate lifting scales, and blue arrows indicate loss of and/or damage to the hair cuticle. Color figure available online.

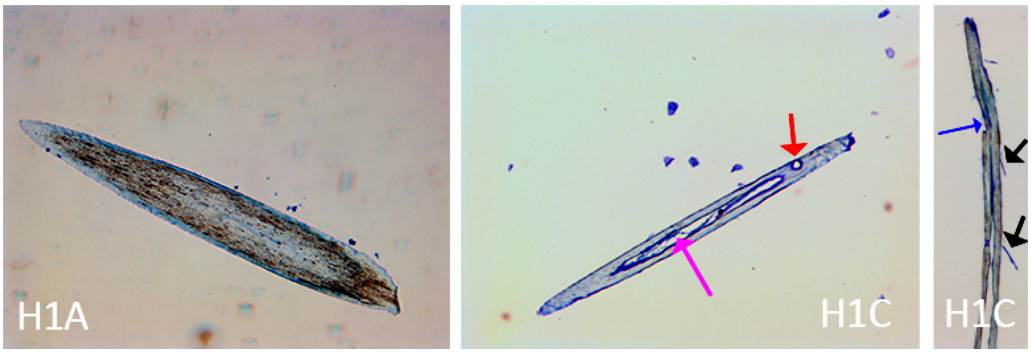


Fig. 2. HRLM images of hairs from Donation 1. The difference in time between collection of samples A and C is 219 days. Left: cross section, magnification 100×. Middle: cross section, magnification 50×. Right: longitudinal section, magnification 50×. Purple arrow indicates hollow cross section of the hair shaft, red arrow indicates fungal holes, black arrows indicate lifting scales, and blue arrow indicates loss of and/or damage to the hair cuticle. Color figure available online.

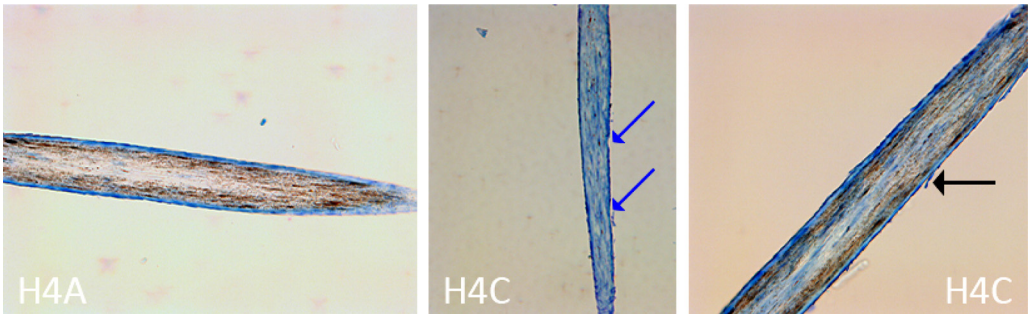


Fig. 3. HRLM images of cross-sectioned hairs from Donation 4. Difference in time between collection of samples A and C is 28 days. Left: cross section, magnification 200×. Middle: cross section, magnification 100×. Right: cross section, magnification 200×. Blue arrows indicate small portions of missing cuticles, black arrow indicates lifting scales coming loose from the cuticle. Color figure available online.

Table 2
H–Pb–Sr isotope data.

Donation	Sample ID	δ^2H (‰)	SD	$^{87}Sr/^{86}Sr$	2SE	[Pb] in ng	$^{206}Pb/^{204}Pb$	2SE	$^{207}Pb/^{204}Pb$	2SE
1	H1A	−52.3	1.9	0.712518	0.000077	0.9	18.631	0.001	15.616	0.001
	H1B	−56.4	1.3	0.708850	0.000019					
	H1C	−43.3	0.7	0.711417	0.000043	9.3	19.258	0.000	15.683	0.000
2	H2A	−78.0	0.7	0.708446	0.000014	0.8	18.218	0.004	15.672	0.003
3	H3A	−57.4	2.2	0.708610	0.000026	0.5	18.584	0.001	15.635	0.001
	H3C	−46.3	1.3	0.711140	0.000015					
4	H4A	−65.3	1.7	0.708179	0.000011	1.2	18.042	0.002	15.504	0.002
	H4B	−60.1	1.2	0.708961	0.000011					
	H4C	−53.6	1.9	0.710024	0.000053	6.0	18.888	0.000	15.657	0.000

Donation	Sample ID	$^{208}Pb/^{204}Pb$	2SE	$^{208}Pb/^{206}Pb$	2SE	$^{207}Pb/^{206}Pb$	2SE	$^{207}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
1	H1A	38.285	0.002	2.055	0.000	0.838	0.000	1.193	2.452
	H1B								
	H1C	38.799	0.000	2.015	0.000	0.814	0.000	1.228	2.474
2	H2A	38.167	0.008	2.095	0.000	0.860	0.000	1.162	2.435
3	H3A	38.373	0.002	2.065	0.000	0.841	0.000	1.189	2.454
	H3C								
4	H4A	37.843	0.005	2.098	0.000	0.859	0.000	1.164	2.441
	H4B								
	H4C	38.521	0.000	2.039	0.000	0.829	0.000	1.206	2.460

frequent fungal holes. In contrast, relatively little difference was observed between the “A” and “C” samples from donation 4. H4C samples showed a slightly higher frequency of lifting scales and loss and/or damage to the cuticle than the H4A intake sample (Fig. 3).

7.2. Isotopic analysis

The results of the H-Sr-Pb isotope analyses are presented in Table 2 and Figs. 4–6. The δ^2H values of the intake “A” samples vary significantly and, except for Donation 4, do not match the predictedH isotope ratios of human scalp hair in Texas [19], despite the fact that all donations spent their last few months to years of life in Texas. Compared to the “A” samples, the δ^2H values of the “B” and “C” samples from donations 1, 3 and 4 all increase during the course of the study, from between +9.0 ‰ in donation 1 (open pit) to +11.7 ‰ in donation 4 (open pit). The isotopic shifts in H in the open pit burials (donations 1 and 4) occur within a week after initial placement. No samples were taken during active decomposition from donations 2 and 3, since these were buried. Consequently, no

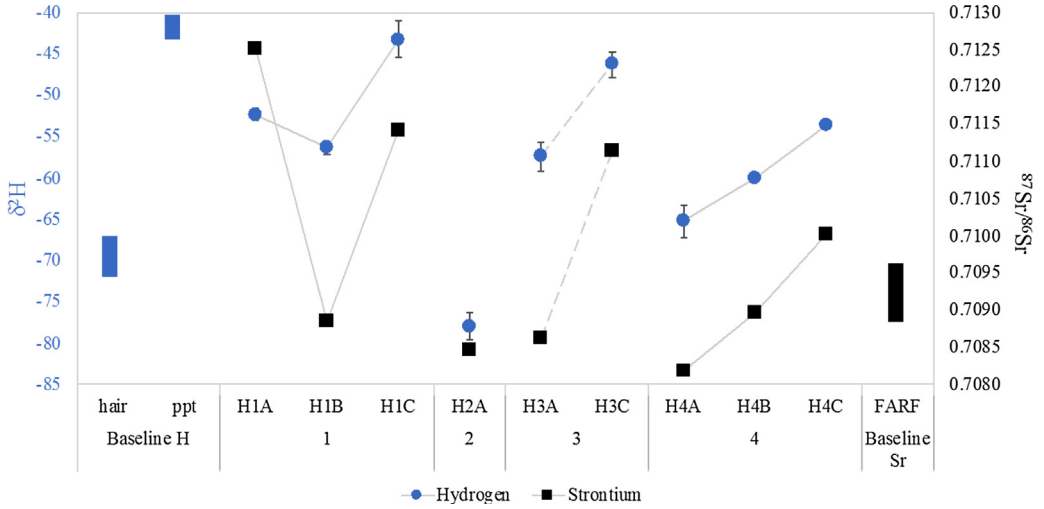


Fig. 4. Mean δ^2H values (triplicate analysis) and standard deviation (1SD, error bars), and $^{87}Sr/^{86}Sr$ ratios of hair samples from 2 open pit (donations 1 and 4) and 2 shallow buried (donations 2 and 3) placed at FARF. $^{87}Sr/^{86}Sr$ 2SE error bars are the same size or smaller than the symbols. Local Sr isotope range at FARF from [9], predicted average δ^2H_{hair} and $\delta^2H_{precipitation}$ values in Texas from [19] and [14] respectively. Color figure available online.

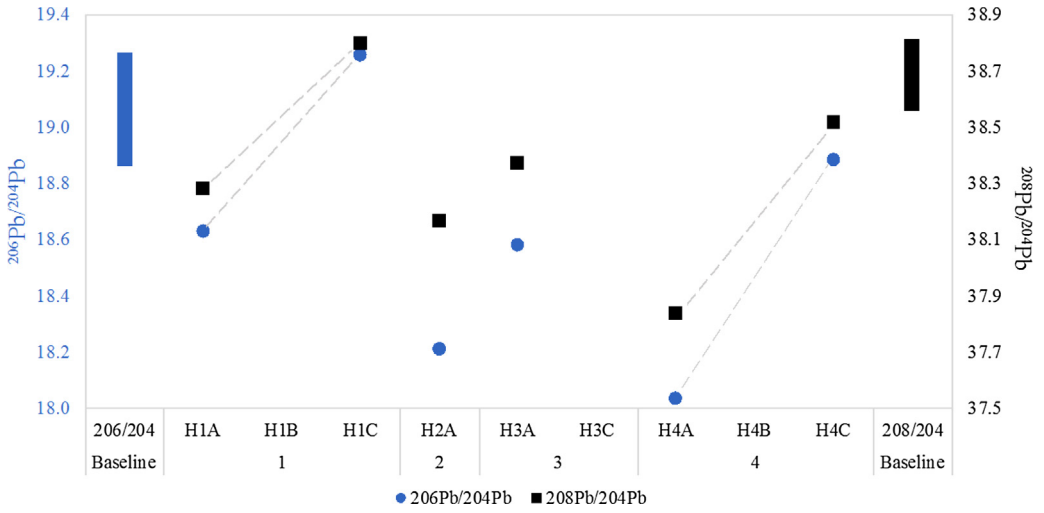


Fig. 5. $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ isotope ratios of hair samples from 2 open pit (donations 1 and 4) and 2 shallow buried (donations 2 and 3) placed at FARF. Error bars are the same size or smaller than the symbols. Local $^{206}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ isotope ratios for FARF soil from [9] (mean ± 1SD). Color figure available online.

constraints can be put on the time that it took for the isotopic shift to occur in simulated clandestine burial contexts. The isotopic signature of donation 1 (open pit) shows a slight isotopic change of −4.1 ‰ between in intake "A" sample and the "B" sample, but then increases significantly from −56.4 ‰ to −43.3 ‰. In donation 4 (open pit), a more linear increase in δ^2H isotope ratios is visible (−65.3 to −60.1 ‰ in the first 6 days, then to −53.6 ‰ in circa 7 months).

In the open burial contexts (donations 1 and 4), the "A" and "C" sample show increased $^{206/207/208}Pb/^{204}Pb$ ratios (Table 2 and Fig. 5), and decreased $^{207/208}Pb/^{206}Pb$ ratios within 4 weeks after placement (donation 4). Unfortunately, both "B" samples and the H3C sample did not provide accurate and reliable Pb isotope data. The isotopic differences between the "A" and "C" samples (ΔPb_{A-C}) are greater in willed-donated body 4 compared to donation 1. On the contrary, the time periods between the samples is greater in body 1 than body 4 (219 and 28 days respectively), suggesting that ΔPb_{A-C} is time-independent.

A similar trend is evident in the Sr isotope data. Within a week after placement, the $^{87}Sr/^{86}Sr$ ratios of the hair samples from donations 1 and 4 respectively decrease and increase significantly compared to initial composition (ΔSr_{A-B}: −0.0037 and +0.00078 respectively). Both samples ultimately exhibited comparable $^{87}Sr/^{86}Sr$ ratios (0.70885 for donation 1 and 0.70896 for donation 4). The H1C and the H4C open pit samples exhibit even more radiogenic $^{87}Sr/^{86}Sr$ ratios compared to the "B" samples (*e.g.*, donation 4 $\Delta^{87}Sr/^{86}Sr_{A-B}$: +0.0011 after 21 days). Nevertheless, the obtained $^{87}Sr/^{86}Sr$ ratio of sample H1C was lower than the biogenic $^{87}Sr/^{86}Sr$ ratio of the intake sample H1A.

Elemental concentration Sr–Pb data indicated that exposed hair samples all contained much less Sr than baseline samples, whereas Pb concentrations increased after burial exposure (Table 2).

8. Discussion

8.1. Microscopy

Clear differences in the degree of hair fiber degradation were observed between the "A" and "C" samples of donations 1 and 4 (Fig. 4). In both samples, the physical degradation increases over

time, which can be attributed to taphonomic processes (including weather-damage) that occurred within the deposition environment throughout the study. The degradation features present in the intake sample of donation 1 (H1A) is most likely related to antemortem exposure to environmental factors, such as sunlight, air pollution, wind, and salt water [47]. The difference in degree of degradation between the H1A and H1C samples is much larger than the difference observed between the H4A and H4C samples. This can be attributed to total time of exposure in this donation (more than 7 months *versus* 3 weeks), as well as to two extreme precipitation events. During these events, both deposition pits filled with water. However, donation 1 remained submerged for a longer period of time compared to donation 4 (see Supplementary data), which likely contributed to the increased frequency of severe types of hair degradation in the H1C samples.

Although SEM and HRLM analyses show differences in the degree of degradation between samples H1C and H4C, the magnitude and direction of the change of the H–Sr–Pb isotope compositions between the intake "A" and the final "C" samples are comparable. Consequently, although the sample size used in this study is limited, these results provide no evidence of a correlation or causal link between isotopic change and the physical change or taphonomic alterations of the hair cuticles and medullae. Therefore, microscopic indicators of hair integrity are no guide to isotopic integrity: microscopic inspection cannot be used to select hair samples for isotopic analysis.

8.2. Isotopic analysis

The H–Pb–Sr isotope data show clear differences between pre- and post-exposure samples. None of the "B" and "C" samples exhibited the biogenic isotope ratio of the unaltered "A" samples. Since previous studies suggest that different factors can affect retrieval of the biogenic endogenous isotope ratios, including environmental factors [9,48] and laboratory protocols [27,29], the question is to what extent the observed differences in this study can be attributed to decomposition, hair degradation, environmental factors, or the drying and cleaning methodology.

The δ^2H values of the "B" and "C" samples in donations 1, 3 and 4 increased toward the interpolated averaged H isotope ratios of precipitation in Texas (−43 ‰ to −19 ‰: Fig. 4 in [14]). The observed δ^2H shift could be interpreted as contamination by local precipitation in central Texas. It could also reflect contamination with evaporatively enriched local environmental water sources (*e.g.* in soil). However, use of a more aggressive acid-leaching protocol [9,29] by adding an HCl leaching step has indicated that H isotope ratios comparable to the antemortem biogenic δ^2H values can be recovered. The observed shifts in H isotope composition presented in this study are therefore conservatively considered to be a consequence of the cleaning methods. Further work is required to substantiate this conclusion.

The Pb concentrations of donations 1 and 4 increased by a factor of 10 and 5 respectively, which is related to the exposure to environmental contaminants. Fig. 7 puts the obtained Pb isotope signatures in environmental context. Previously published isotope data from dental enamel has values intermediate between geological/ore and atmospheric/pollution sources. The hair data in this study has similar values (samples 1A and 3A) or shows values more dominated by pollution (2A and 4A). This biogenic/atmospheric signal was overtaken by an exogenous local geogenic signal within 4 weeks: the Pb isotope ratios of both "C" samples shift toward the local FARF environmental (soil) signatures. This parallels the published soil leachate data given in Gordon et al. [9] (Figs. 5 and 6).

The biogenic antemortem $^{87}Sr/^{86}Sr$ ratios of hair keratin are changed within days of initial exposure. There was heavier than usual precipitation at FARF in the days prior to the collection of the "B" samples from donations 1 and 4. Although the $^{87}Sr/^{86}Sr$ ratio of precipitation can vary significantly within the research facility's region [9], and precipitation water was not collected during the course of this actualistic study, it is plausible that both "B" samples recorded a meteoric signal. The difference between B and C samples' isotopic ratios may be due to (1) differences in deposition rates of Sr fractions from different environmental sources during exposure, 2) differences in removal efficiency of Sr fractions from different environmental sources during washing, or (3) a combination of the two. The obtained Sr isotope compositions thus indicate that external contaminants, permanently overtook the irretrievable biogenic Sr isotope signature. Previous research underlined the susceptibility of Sr in hair keratin to exchange withsurrounding matrix and the difficulty of the complete removal of exogenous Sr [7,9,29,30]. An aggressive washing methodology of the sample hairs, using 0.1 M HCl leaching steps as suggested by Tipple and colleagues [29], was not included in this study (but see [7]). As a result, it is likely that external Sr contamination was not

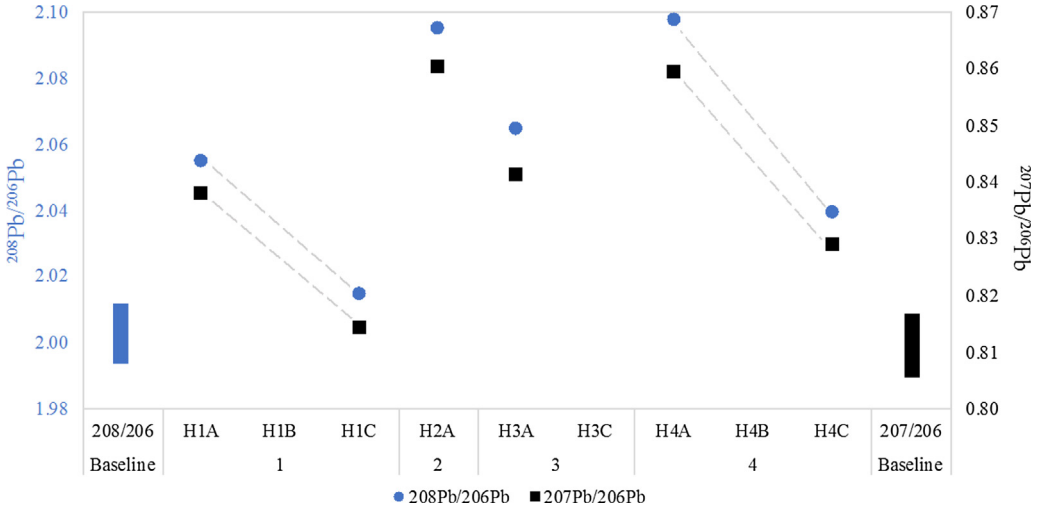


Fig. 6. $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ isotope ratios of hair samples from 2 open pit (donations 1 and 4) and 2 shallow buried (donations 2 and 3) placed at FARF. Error bars are the same size or smaller than the symbols. Local $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ isotope ratios for FARF soil from [9] (mean ± 1SD). Color figure available online.

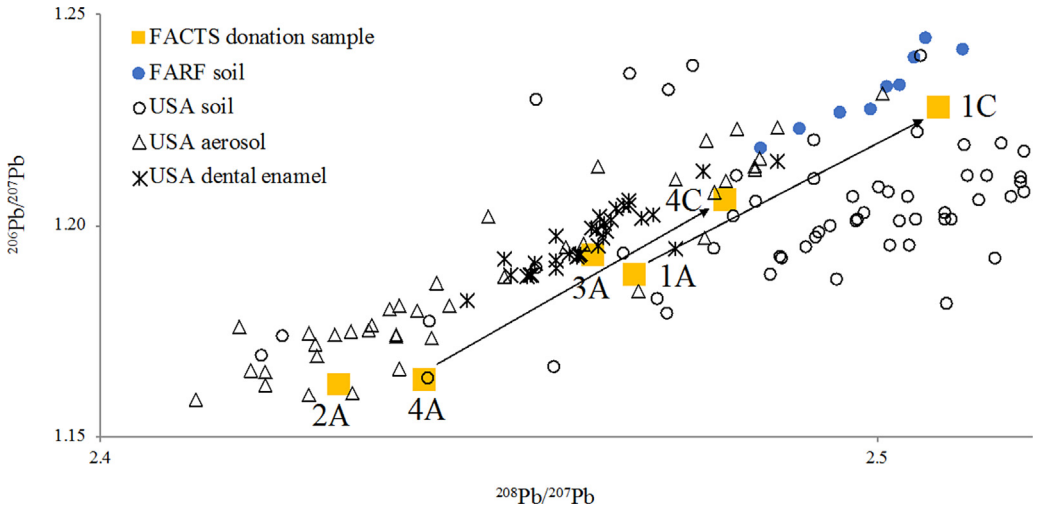


Fig. 7. $^{208}Pb/^{207}Pb$ and $^{206}Pb/^{207}Pb$ isotope ratios of hair samples from 2 open pit (donations 1 and 4) and 2 shallow buried (donations 2 and 3) placed at FARF plotted against modern human dental enamel data from the USA [49,50], USA atmospheric [51,52], USA soil [53], and FARF soil data [9]. The complete soil $^{208}Pb/^{207}Pb$ and $^{206}Pb/^{207}Pb$ range from [53] ranges from 2.43 to 2.86 and 1.16 to 1.31 respectively (Supplementary data 3). Error bars are the same size or smaller than the symbols.

completely removed. However, Sr concentration in this study show a significant decrease in [Sr] in the hairs between the "A" and "C" samples of between 80%–94%. Hence, the weak cleaning protocol applied in this study did leach out most of the Sr, but clearly was unable to remove all diagenetic Sr. It is suggested that the observed alteration in isotopic value during burial cannot simply derive from the absorption of exogenous mineral into or onto the hairs, but implies that the majority of the original Sr became more labile during decomposition, possibly due to changes in bonding sites [54].

9. Conclusions

The data from this study show that biogenic H–Pb–Sr isotopic ratios in scalp hair are rapidly altered after deposition in a burial environment, either open or closed. Sr and Pb isotopic values indicate the incorporation of exogenous Sr and Pb into the hair, within a week of exposure. Though this study was small, the results suggest that time of exposure and the origin of the contaminant material (geogenic vs. climatic) significantly affect observed isotope values.

The data presented here are artefacts of both cleaning protocol and taphonomic processes. Antemortem biogenic H-Pb-Sr isotopic ratios are challenging to recover, as shown by Saul [7] and Gordon et al. [9]. This emphasizes the need for thorough testing and validation not only of laboratory protocols but of the full forensic methodology (*e.g.*, burial modality and duration, body completeness, recording of climatic data, adequate sampling of environment) before isotopic data interpretation.

The microscopic assessment of the hair samples provides no evidence of a relationship between isotopic alterations and the physical state of preservation of the hair cuticles and medullae. SEM and HRLM inspection cannot be used to select forensic hair samples for isotopic analysis.

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CRediT authorship contribution statement

Lisette M. Kootker: Investigation, Formal analysis, Writing - original draft. **Isabella C.C. von Holstein:** Investigation, Methodology, Writing - review & editing. **Jelle Broeders:** Investigation, Formal analysis, Data curation, Writing - original draft. **Daniel J. Wescott:** Data curation, Resources, Writing - review & editing. **Gareth R. Davies:** Funding acquisition, Resources, Writing - review & editing. **Hayley L. Mickleburgh:** Conceptualization, Funding acquisition, Project administration, Writing - original draft, Data curation, Supervision, Resources.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2020.110336.

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