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Extraction of Human DNA from Soil in a Simulated Clandestine Grave

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Extraction of Human DNA from Soil in a Simulated Clandestine Grave

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ABSTRACT

Locard's Exchange Principle states that "every contact leaves a trace." The same principle applies when a perpetrator of a homicide decides to bury a victim in a clandestine grave. If a perpetrator originally buried a murder victim in haste to prevent discovery and then decided to later move the victim's body to a remote location for disposal, decomposition fluids from the victim's body (which contain cellular material and therefore DNA) would remain in the surrounding grave soil at the original burial site. It is possible that investigators could: 1) prove that a human body had once laid in that location (as opposed to animal remains), and 2) determine the identity of the victim via forensic DNA typing of the cellular remnants and body fluids left behind in the soil.

In this case study, a simulated clandestine grave was created in the laboratory using human remains (femur) from a previous cemetery exhumation. The remains were donated for research and had not been embalmed prior to burial. Over a 4-week period, the remaining soft tissue attached to the femur was allowed to decompose naturally; soil samples from directly underneath each femur section were collected at 1-week, 2-week, 3-week, and 4-week intervals. Two different DNA extractions methods (silica-based and organic) were performed in an attempt to recover human DNA from decomposition fluids in the soil. DNA quantities recovered from each soil sample aliquot were determined using a human-DNA-specific quantification kit and real-time (quantitative) PCR. The organic extraction method yielded higher amounts of human nuclear DNA for downstream STR genotyping than silica-based DNA extraction. The average DNA quantities (ng) recovered using organic and silica-based DNA extraction were 1.6929 ng and 0.0445 ng, respectively. Although attempts were undertaken to purify DNA and remove PCR inhibitors from the soil (e.g., humic acids, fulvic acids), qPCR results indicated that many samples were still exhibiting signs of inhibition. Although this study demonstrates proof-of-concept that human DNA can be recovered from decomposition fluids in soil underneath human remains in clandestine graves, future research efforts should focus on improving DNA extraction approaches that would better facilitate removal of soil-derived inhibitors.

INTRODUCTION & LITERATURE REVIEW

Paleoanthropology and origins of forensic taphonomy

In the early 1940s, early pioneers in the field of paleontology embraced the term 'taphonomy' presented by Ivan Efremov, a Soviet paleontologist. Originally, taphonomy was solely focused on the areas of what happened to an organism post-burial, the conversion of bone to fossil, and preservation issues. It was not until the 1970s when concepts of taphonomy spilled into paleoanthropology where taphonomic concepts were used in examining bone weathering to "give specific information concerning surface exposure of bone prior to burial and the time periods over which bones accumulated" [1]. During this decade, forensic anthropologists (who were primarily concerned with providing accurate reconstructions of biological profiles of individuals represented by skeletal remains found in forensic settings) noticed a gap in paleoanthropology and forensic anthropology regarding contextual grounding of determinations of time since death and specific reasons for scattered, missing, and damaged remains. Forensic anthropologists attempted to address this lack of knowledge and similar questions by directing themselves to a taphonomic perspective with the creation of forensic taphonomy [1]. In short, forensic taphonomy is the application of the laws of burial relating to the transformation of organic material in a given environment following the cessation of life regarding the determination of postmortem interval (PMI) and the assignment of cause/manner of death [2]. Forensic taphonomy is also related to the deceased organism's reconstruction of their biology or ecology as well as the reconstruction of the circumstances of their death [1].

The difference between taphonomy in paleontology and forensic taphonomy is that forensic taphonomy is primarily concerned with what happened to the body immediately after death until recovery [1]. In other words, the focus lies within the scope of the human decomposition process.

Stages of human decomposition

Traditionally, postmortem human decomposition is marked by six stages that have been largely assigned through subjective analysis and reasonable indication of physiochemical changes in decomposing remains. The six stages of human decomposition are noted as fresh, bloated, active decay, advanced decay, dry, and remains [1,2].

Immediately upon death, the body enters the fresh stage. This first stage consists of autolysis, which is characterized by widespread cellular death (i.e., apoptosis or necrosis) due to cessation of ATP production followed by domination of anaerobic processes [3]. The body's prolonged depletion of oxygen permits microbiota in the gastrointestinal (GI) tract to flourish and further the decay process via putrefaction during the Bloated Stage. [2]. Putrefaction is when the anaerobic splitting of proteins by bacteria and fungi leads to the formation of foul-smelling, incompletely oxidized products [3]. Furthermore, this process results in color changes of the torso (e.g., "Green Belly") and distention (swelling) of the bowel from the accumulation of gases [2,3]. Due to accumulation of gases inside the body, an initial purging of cadaveric fluids from orifices into the surrounding environment occurs [3]. This purging persists and increases during the next two stages of active decay and advanced decay [3]. When a body is in active decay, the persistent purging coincides with occurrences of skin slippage and breakage on the body [2]. Additionally, continuous purging leads to a substantial amount of cadaveric material entering the soil, thus creating a **Cadaveric Decomposition Island (CDI)** that is characterized by significant change in the surrounding soil ecology [2]. Other features of active decay tend to involve cadaveric mass loss and increased maggot activity; however, migration of maggots from the cadaver indicates continuation to the advanced decay stage [2]. During this fourth stage, the CDI is associated with the highest levels of nutrient-rich materials from purging that leads to an area of increased soil carbon, nutrients, and pH levels [2,3]. After advanced decay, the dry stage consists of remnant tissues losing any remaining moisture. The final stage of decomposition occurs when the cadaver has lost all remaining flesh and soft tissue, resulting in complete skeletonization of the remains. Also, during this final stage, the surrounding environment will revert to its original state except for the soil underneath the cadaver [2].

While these are the classical six stages of human decomposition, it should be clarified that the general progression of decomposition is highly variable due to abiotic extrinsic factors (e.g., ambient temperature, acidity of the soil, availability of moisture, the partial pressure of oxygen), and biotic extrinsic factors (e.g., microbes, arthropods, large scavengers). Both abiotic and biotic extrinsic factors can significantly affect the progression of decomposition [4].

Preservation and persistence of DNA in soil during and after human decomposition

Due to continuous purging during the decomposition stages of bloated, active decay, and advanced decay, human DNA in cadaveric fluid settles in the surrounding soil, particularly directly underneath the body. When human DNA settles in the cadaver's surrounding environment, it becomes vulnerable to "both cellular enzymes from the cadaver itself and extracellular enzymes from increased microbial activity" [3]. It has been shown that DNA can persist in soil for thousands of years, although its persistence depends on environmental factors associated with the soil in question [2,5]. Among these factors include soil texture, water content (which promotes microbial activity), nutrient diffusion, soil acidity, and hydrolytic enzyme activity [3]. It should be noted that preservation/persistence of DNA in both soil and cadaveric fluid is thought to be due to nullification (or mitigation) of environmental destructive mechanisms via adsorption of DNA to the matrix of certain soil types (e.g., sand, clay, silt) as well as to compounds within the soil (e.g., humic compounds) [3,5]. This direct interaction between DNA and soil matrices/components provides a protective barrier for DNA, shielding the DNA from nucleases and other enzymes in the soil environment [3].

Humic compounds in soil and challenges with PCR inhibition

While DNA preservation as a result of binding to humic compounds (e.g., humic acid, fulvic acid) in the soil environment provides a potential avenue of confirming the location of previous clandestine burial sites, recovery of DNA from soil samples can be challenging because humic compounds are known inhibitors of the Polymerase Chain Reaction (PCR) and often co-extract with the DNA of interest [6]. Humic compounds are dark-colored, amorphic, highly stable compounds that are produced by the Maillard reaction, a biochemical aging process that involves non-enzymatic browning reactions between amines and carbonyl compounds, specifically glucose or glucose 6-phosphate (G-6-P) [6]. One study found that, depending on the soil type, humic compounds can comprise 5.0 mg/g − 7.63 mg/g of soil [6]. Another study demonstrated that these compounds can be extracted from soil via alkylation and then fractioned into humic acid (HA) and fulvic acid (FA) fractions via acidification [6]. Both HA and FA have exhibited charge-to-mass ratio similarities to DNA and structural heterogeneity, which has led to the conclusion that humic compounds are one of the most recalcitrant impurities during DNA isolation

procedures [6]. Furthermore, HAs are noted to have a negative charge as well as similar physiochemical properties to the phosphate groups within the sugar-phosphate backbone of DNA [6]. This indicates that HAs can compete with DNA for adsorption sites during DNA purification steps [6]. Consequently, real-time PCR (qPCR) studies have indicated that the inhibition mechanism of HAs can be classified as non-competitive, i.e., in which the inhibitor binds to the enzyme-substrate complex, making it unreactive (although non-competitive inhibition can be reversed by removal of the inhibitor during DNA extraction) [6]. Removal of humic compounds (i.e., the inhibitor) from its bond to DNA (i.e., the enzyme-substrate complex) during DNA extraction does allow for successful DNA amplification via PCR [7]. This indicates that the particular DNA extraction method used for cadaveric soil samples is very important and should be selected based on its ability to effectively remove (or greatly reduce) the quantity of humic compounds (primarily HAs) present.

Relevant case studies

In a 2017 study performed by the University of Tennessee's Anthropology Research Facility (ARF), researchers assessed the relative persistence of human DNA in soil over the course of decomposition [3]. In this study's methodology, DNA was isolated from soil samples using the Powerlyzer PowerSoil® DNA Isolation Kit (MOBIO), using a modified manufacturer's protocol in which chemical lysis was used to increase the ratio of human to bacterial DNA [3]. This DNA extraction kit includes a set of solutions that are effective at removing PCR inhibitors (e.g., HAs) when the procedure is performed correctly [3]. In the University of Tennessee study, removal of soil-derived PCR inhibitors was successful for 27-out-of-45 DNA samples and researchers were able to recover mitochondrial DNA (mtDNA) from the soil throughout all stages of decomposition; however, nuclear DNA was not recovered for most of the samples [3]. Recovery of mtDNA likely occurred due to its circular structure and location within double-membrane-bound organelles, which provided protection from nuclease degradation [7]. Furthermore, the chances of recovering mtDNA from human remains is higher than nuclear (autosomal) DNA because there are inherently greater numbers of mtDNA copies per human cell (i.e., hundreds-to-thousands) in comparison to only two copies of nuclear DNA per cell [3,7]. Additionally, higher amounts of mtDNA were able to be recovered during the later stages

of decomposition because of increased amounts of cadaveric fluid entering the soil during the process of purging [3]. Overall, this study demonstrates that once the issue of humic compound inhibition is removed, the ability to extract and sequence mtDNA becomes possible (and even probable).

Another study in 2017 involved searching for traces of hominin DNA at archaeological sites previously known to be locations of mass hominin assemblage, yet the physical human remains were not present at the site(s) [8]. Researchers collected 85 soil samples from seven archaeological sites with known hominin occupation, varying in age between approximately 14 thousand and 550 years ago [8]. Soil samples were subjected to DNA isolation, purification, and amplification using protocols that asserted the ability to remove PCR inhibitors (including humic compounds) [8]. Post-purification, amplified DNA samples were converted into sequencing libraries and hybridization capture [a method of targeted next generation sequencing (NGS) that uses long, biotinylated oligonucleotides baits (probes) to hybridize regions of interest] was performed [8]. This process allowed researchers to effectively retrieve Neandertal and Denisovan mtDNA from many Late and some Middle Pleistocene cave soil samples [8]. This study demonstrates that soil can preserve DNA from archaeological samples.

Moreover, in a recent 2021 study under the Lundbeck Foundation GeoGenetics Centre and other research institutions, researchers sequenced environmental DNA (eDNA) fragments derived from shed cells, hair, feces, and urine preserved within sediment to determine if it is feasible to retrieve genome-wide data directly from ancient eDNA [9]. Researchers recovered DNA from a total of 48 sediment subsamples within three stratigraphic layers from cave sediment samples obtained from the Chiquihuite Cave in the Astillero Mountains of North México [9]. The extracted ancient eDNA was converted into 65 libraries for Illumina® shotgun sequencing and then used for competitive mapping against the Reference Sequence (RefSeq) mitochondrial database (Version 92) since eDNA is restricted to organellar DNA (mitochondrial and chloroplast) [9]. Results indicated to researchers that it is possible to analyze DNA from environmental samples in a similar manner as is currently done for DNA from fossil remains to track the evolutionary lineage between the American and Mexican Black Bear [9]. Furthermore, this recent study demonstrates that recovery of DNA from soil has been applied to cases involving animal

lineage and evolution with mtDNA. However, there are still a lack of studies pertaining to the recovery of nuclear (autosomal) DNA from soil for forensic applications.

Purpose and significance of the current study

Locard's Exchange Principle states that every person who is physically involved in a crime leaves some minute trace of their presence and often takes something away [10]. This principle was demonstrated in the case studies described previously since contact between the cadaver and the soil environment (as well contact between the hominins' or ancient animals' assemblage and soil) led to the discovery of being able to isolate mtDNA for identification purposes [3,8,11]. Although mtDNA can be informative, it is a maternal lineage marker that does not possess the same discriminatory power as nuclear (autosomal) DNA. On its own, mtDNA can only serve to associate human remains with a particular familial line and cannot be used to make a positive identification.

The primary goal of the current study was to extract human nuclear (autosomal) DNA from soil samples collected from underneath decomposing human remains in a clandestine burial site. If human nuclear (autosomal) DNA can be recovered in sufficient quantities for STR analysis, this study could serve as a primer for forensic investigation of suspected homicides and clandestine burial sites, both to confirm the previous presence of a decomposing body and to ultimately make a positive identification of the victim in the case. This would be a significant contribution in attempts to assist with the investigation of the 15,000 homicides and over 100,000 active missing person cases that occur in the United States annually [12].

MATERIALS & METHODS

Phase I: Simulated clandestine burial of decomposing human remains

A clandestine grave or dump site (surface deposition) was simulated in a laboratory hood at the University of New Haven, Department of Forensic Science (Dodd's Hall). A square glass container was filled with soil from Litchfield County, Connecticut USA. Prior to filling with soil, the glass container was pre-sterilized via soaking in Tergazyme® Enzyme Active Detergent (Alconox Incorporated, White Plains, New York) and sodium hypochlorite (NaOCl, bleach) for 30 minutes. After soaking, the container was thoroughly rinsed with molecular grade water and allowed to dry in a dead-air hood.

Human remains (i.e., a section of femur) were donated for this research project after a prior cemetery exhumation was conducted for a private forensic case. The body was exhumed at the 9-month postmortem interval (PMI) and was in an advanced state of decomposition (i.e., soft tissue still remaining, not completely skeletonized). Due to the family's cultural and religious customs, embalming had not been performed prior to burial. This was an important consideration for use of these remains in the current study, as embalming chemicals (e.g., formaldehyde) are destructive to endogenous DNA. Samples were anonymized upon collection, with no personal identifying information recorded that could link the identity of the decedent to this study. This research project met the criteria for exemption under 45 CFR 46.104 b4 (i.e., collection or study of existing pathological or diagnostic specimens, where the information is private but identifiers are not recorded by the researcher). Exempt status was granted by the University of New Haven's Institutional Review Board (IRB), Protocol # 2021-013.

Six human femur sections with adhered soft tissue (A-F) were embedded in soil and allowed to decompose for a four-week time period (February – March 2021) at room temperature in a laboratory at the University of New Haven's Department of Forensic Science (**Figure 1)**. Moisture content of the soil was maintained over the entire 4-week study period using a sterile, 50-mL polypropylene conical tube and molecular grade water. Researchers wore appropriate personal protective equipment (PPE) to mitigate potential biohazard exposure and to prevent cross-contamination with exogenous DNA from the researchers and other laboratory personnel.

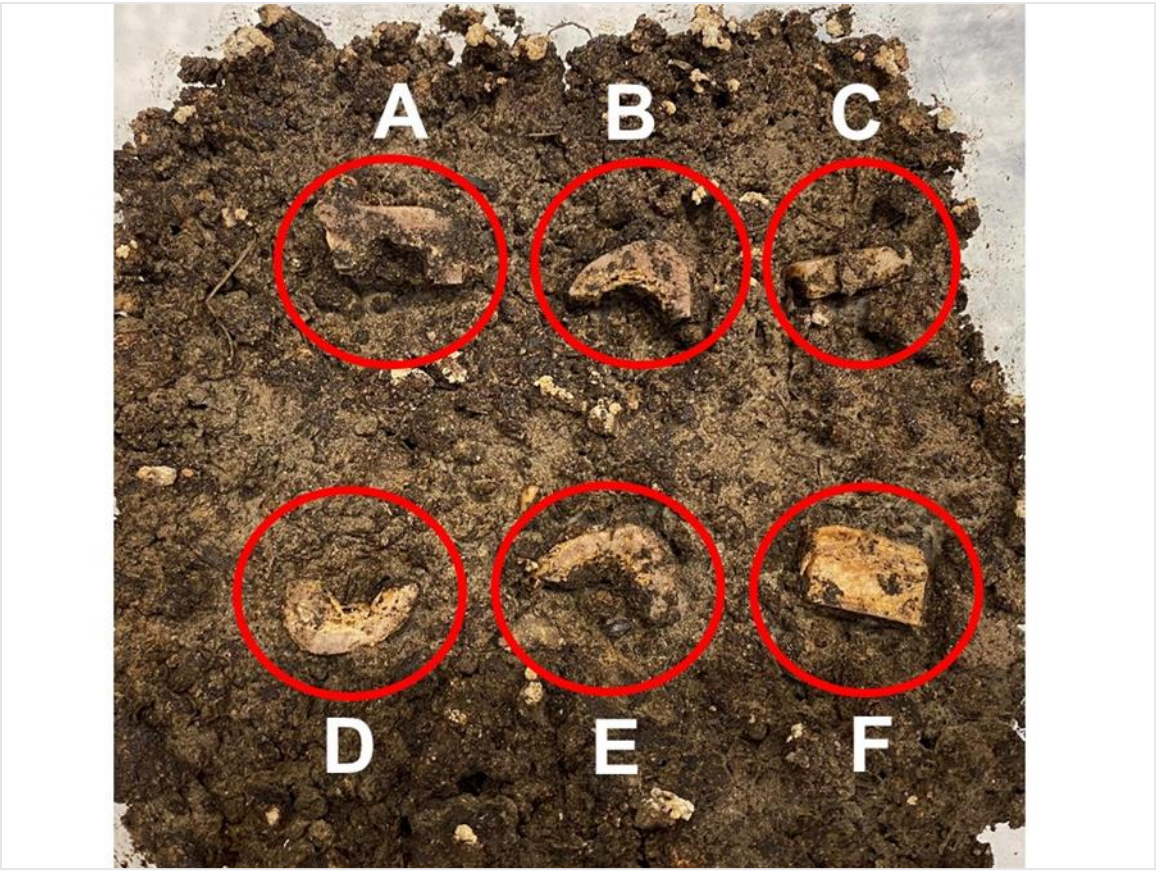

Figure 1. Simulated clandestine grave containing six (6) femur sections with adhered decomposing soft tissue (labeled A-F), embedded in soil from Litchfield County, Connecticut USA (pH = 5) for a period of 4 weeks.

Phase II: Collection of soil samples from simulated clandestine grave

Soil samples were collected from the **Cadaveric Decomposition Island (CDI)** directly underneath each decomposing femur section (A-F) at 1-week, 2-week, 3-week, and 4-week time intervals. To collect the soil samples, the researcher (while wearing sterile gloves) used one hand to lift the femur section while the other hand scooped soil using a sterile 1.5-mL microcentrifuge tube. A total of 24 soil samples were collected (four per time interval, for each of the six femur sections). Post-collection, each soil sample was stored at -20°C to reduce further nuclease activity and to preserve any human DNA present prior to initiation of DNA extraction procedures. The pH of the soil was measured in triplicate using Fisherbrand® pH paper (pH = 5).

<u>Phase III</u>: Recovery of human DNA from soil samples

DNA extraction methods

In preparation for DNA extraction, soil samples collected from the simulated clandestine grave were separated into 200-mg aliquots and placed into sterile, pre-labeled 1.5-mL microcentrifuge tubes using disposable (sterilized) plastic spatulas. Sample identification (ID) numbers were generated to designate each femur section's position in the simulated clandestine grave and its respective collection time. For example, Sample A1 = femur section in position A collected at the 1-week time interval; Sample A2 = femur section in position A, collected at the 2-week time interval. A total of seventy-four (74) 200-mg aliquots of soil and twenty-two (22) aliquots of soil with < 200-mg of soil were available to proceed with DNA extraction.

Two different methods of DNA extraction were employed on two sets of 200-mg soil aliquots. The first DNA extraction method used was silica-based DNA extraction using the QIAamp® DNA Investigator Kit (Qiagen Corporation, Germantown, Maryland USA) and the *Isolation of Total DNA from Bones and Teeth* protocol. Procedures were followed according to manufacturer recommendations, using a 25-µL elution volume and with one modification. A 200-mg aliquot of soil from each of the original 24 soil samples, three reagent blanks (RBs), and three 200-mg aliquots of soil from the decedent's original exhumation site were processed instead of 100-mg of bone powder. The second DNA extraction method used was organic DNA extraction using the UNT Center for Human Identification (UNTCHI) Standard Operating Procedure (SOP). A second set of 200-mg aliquots of soil from the original 24 soil samples and two reagent blanks were processed with this protocol, using a 25-µL elution volume. A total of fifty-one (51) samples were extracted for DNA (n=51).

DNA concentration and inhibitor removal

For all soil samples that were processed using the organic DNA extraction method, the aqueous layer from each sample was concentrated using Amicon® Ultra-4 30K Centrifugal Filtration Units (Millipore Sigma, Burlington, Massachusetts USA).

At this stage, because of brown coloration observed in the elutions **(Figure 2)**, each of the fifty-one (51) processed samples and five reagent blanks (RBs) from both DNA

extraction methods were further processed for inhibitor removal using the QIAquick® PCR Purification Kit (Qiagen Corporation, Germantown, Maryland USA), according to manufacturer recommendations.

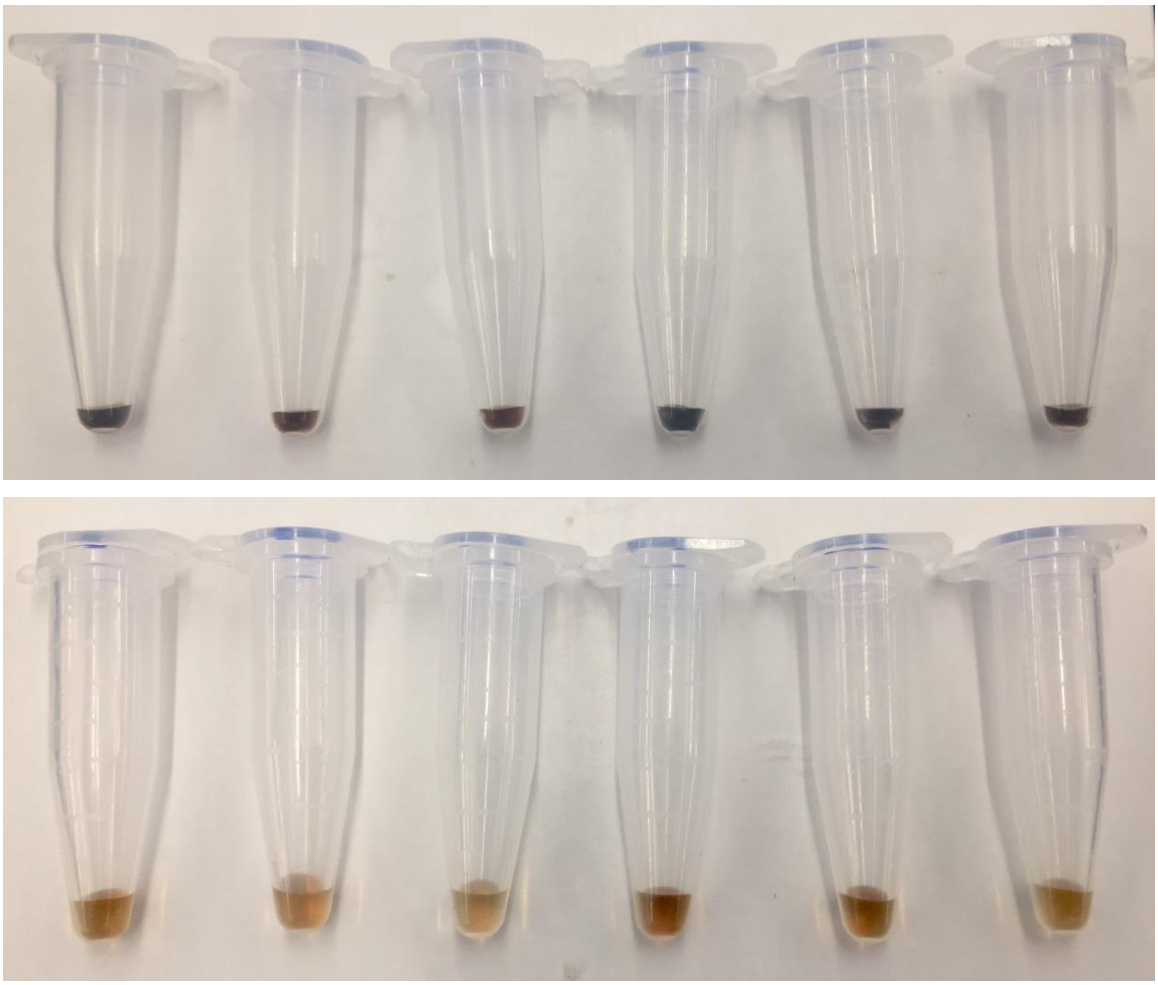

Figure 2. Examples of brown coloration observed in soil sample elutions immediately after DNA extraction *(top)* and after inhibitor removal attempts *(bottom)* using the Amicon® Ultra-4 30K Centrifugal Filtration Units (for samples extracted using the organic method) and QIAquick® PCR Purification Kit (for all samples, regardless of extraction method).

Quantification of human DNA recovered

Post-purification and inhibitor removal, the amount of DNA recovered from each soil sample was determined via quantitative PCR (qPCR) using the Quantifiler™ Trio Human DNA quantification kit (Thermo Fisher Scientific, Waltham, Massachusetts USA) and the QuantStudio™ 5 Real-time PCR System (Applied Biosystems, Waltham,

Massachusetts USA). Due to the brown coloration that persisted after cleanup attempts, two different iterations were explored during qPCR to mitigate the effects of the presence of co-extracted inhibitors (e.g., humic acids, fulvic acids). The manufacturer recommendation of adding 2µL DNA extract to the qPCR reaction was conducted for 30 samples from the simulated clandestine grave (i.e., 6 samples from silica-based DNA extraction; 24 samples from organic DNA extraction) and three samples from the original exhumation site. A 1:1 dilution was then conducted on 48 samples from the simulated clandestine grave (i.e., 24 samples from silica-based DNA extraction; 24 samples from organic DNA extraction) to mitigate the effects from the co-extracted inhibitors, reducing the manufacturer recommended 2-µL input volume by half. For clarification, the second iteration that was attempted to mitigate the presence of inhibitors involved mixing 1-µL DNA extract and 1-µL of molecular grade water, for a total input of 2-µL volume to the qPCR reaction.

RESULTS & DISCUSSION

After the initial incubation (cell lysis) step in the silica-based DNA extraction method, extreme care must be taken when transferring the lysate into the silica columns. Carryover of minute soil particles can clog the columns and block DNA-binding sites on the silica membrane. Some samples experienced clogged columns and required multiple centrifugation steps to get all of the lysate to flow through, which may have resulted in sub-optimal DNA-binding and/or loss of DNA. Organic extraction offers a method to isolate DNA without the use of a solid substrate (i.e., column); hence, the same challenges were not encountered with samples processed using this isolation approach. All soil samples processed using either the silica-based or organic DNA extraction methods required a purification step to remove co-extracted inhibitors. The color of an extract can be an indicator that inhibitors are present; all of DNA extracts in this study varied in color from light brown to dark brown, and most retained a brown tint/coloration even after completion of the purification steps. For this reason, each sample was processed during quantitative PCR (qPCR) using the manufacturer-recommended volume (2µL DNA) and a 1:1 dilution (1µL DNA, 1µL molecular grade water). **Table 1** and **Table 2** demonstrate that no human DNA was detected when 2µL of extracted DNA was used for quantification. For these samples, an undetermined (UND) value for the Internal Positive Control (IPC) indicates complete inhibition of the qPCR reaction. Too much inhibitor was present to assess DNA recovery. The extraction reagent blanks (RBs) were free of exogenous (contaminant) DNA.

Sample ID	T.IPC (Cт)	T. Large Autosomal (Cт)	T. Small Autosomal (Cт)	T.Y (Cт)	DNA Concentration (ng/µL)	Total DNA quantity (ng)
A1	UND	UND	UND	UND	UND	UND
E1	UND	UND	UND	UND	UND	UND
B2	UND	UND	UND	UND	UND	UND
F2	UND	UND	UND	UND	UND	UND
C3	UND	UND	UND	UND	UND	UND
D4	UND	UND	UND	UND	UND	UND
RB	27.71176	UND	UND	UND	UND	UND

Table 1. DNA quantification results for silica extracts using the manufacturer-recommended 2-µL input volume. Complete inhibition of the qPCR reaction was observed, as indicated by the UND (undetermined) values for the internal positive control (IPC).

Table 2. DNA quantification results for organic extracts using the manufacturer-recommended 2-µL input volume. Complete inhibition of the qPCR reaction was observed, as indicated by the UND (undetermined) values for the internal positive control (IPC).

Sample ID	T.IPC (Cт)	T. Large Autosomal (Cт)	T. Small Autosomal (Cт)	T.Y (Cт)	DNA concentration (ng/µL)	Total DNA Present (ng)
A1	UND	UND	36.10172	UND	0.003	UND
B1	UND	UND	UND	UND	UND	UND
C1	UND	UND	UND	UND	UND	UND
D1	UND	UND	UND	UND	UND	UND
E1	UND	UND	32.87539	UND	0.0276	UND
F1	UND	UND	UND	UND	UND	UND
A2	UND	UND	UND	UND	UND	UND
B2	UND	UND	UND	UND	UND	UND
C2	UND	UND	UND	UND	UND	UND
D2	UND	UND	UND	UND	UND	UND
E2	UND	UND	UND	UND	UND	UND
F2	UND	UND	UND	UND	UND	UND
A3	UND	UND	UND	UND	UND	UND
B3	UND	UND	UND	UND	UND	UND
C3	UND	UND	UND	UND	UND	UND
D3	UND	UND	35.61272	UND	0.0042	UND
E3	UND	UND	UND	UND	UND	UND
F3	UND	UND	UND	UND	UND	UND
A4	UND	UND	UND	UND	UND	UND
B4	UND	UND	UND	UND	UND	UND
C4	UND	UND	UND	UND	UND	UND
D4	UND	UND	UND	UND	UND	UND
E4	UND	UND	UND	UND	UND	UND
F4	UND	UND	UND	UND	UND	UND
RB (aa)	27.30763	UND	UND	UND	UND	UND
RB (PL)	27.72183	UND	UND	UND	UND	UND

Table 3 and **Table 4** demonstrate that both DNA extraction methods yielded human DNA from soil samples when only 1-µL of DNA extract was added. Diluting the co-extracted inhibitor enabled the quantification (qPCR) assay to proceed and detect human nuclear (autosomal) DNA.

Table 3. DNA quantification results for silica extracts using a 1:1 dilution (1µL DNA, 1µL molecular grade water) to mitigate the effects of co-extracted inhibitors. Human nuclear (autosomal) DNA was detected in six (6) samples (shown in **blue**). DNA quantities circled in **red** are within the range necessary to detect alleles for the 20 core CODIS STR loci used in forensic DNA casework.

Sample ID	T.IPC (CT)	T. Large Autosomal (CT)	T. Small Autosomal (CT)	T.Y (CT)	DNA Concentration (ng/µL)	Total DNA Present (ng)
A1	34.90591	UND	38.18589	UND	0.0007	0.0341
B1	30.31268	UND	37.52676	UND	0.0023	0.1132
C1	UND	UND	UND	UND	UND	UND
D1	UND	UND	38.54887	UND	UND	UND
E1	34.64321	UND	UND	UND	UND	UND
F1	29.80792	UND	UND	UND	UND	UND
A2	30.98649	UND	UND	UND	UND	UND
B2	28.08595	UND	UND	UND	UND	UND
C2	37.4547	UND	38.61131	UND	0.0011	0.0537
D2	UND	UND	38.23411	UND	UND	UND
E2	UND	UND	UND	UND	UND	UND
F2	28.59355	UND	39.31216	UND	0.0003	0.0151
A3	38.2918	UND	UND	UND	UND	UND
B3	32.09287	UND	UND	UND	UND	UND
C3	28.10959	UND	39.14336	UND	0.0003	0.017
D3	29.96454	UND	UND	UND	UND	UND
E3	28.4497	UND	UND	UND	UND	UND
F3	29.27961	UND	UND	UND	UND	UND
A4	29.64053	UND	UND	UND	UND	UND
B4	28.21565	UND	UND	UND	UND	UND
C4	28.30983	UND	UND	UND	UND	UND
D4	UND	UND	UND	UND	UND	UND
E4	28.33393	UND	39.29567	UND	0.0007	0.0336
F4	28.12598	UND	UND	UND	UND	UND
RB1	27.46618	UND	UND	UND	UND	UND
RB2	27.49621	UND	UND	UND	UND	UND

Table 4. DNA quantification results for organic extracts using a 1:1 dilution (1µL DNA, 1µL molecular grade water) to mitigate the effects of co-extracted inhibitors. Human nuclear (autosomal) DNA was detected in six (6) samples (shown in **blue**). DNA quantities circled in **red** are within the range necessary to detect alleles for the 20 core CODIS STR loci used in forensic DNA casework.

Sample ID	T.IPC (CT)	T. Large Autosomal (CT)	T. Small Autosomal (CT)	T.Y (CT)	DNA Concentration (ng/µL)	Total DNA Present (ng)
A1	30.39119	UND	34.57915	UND	0.0171	0.8571
B1	29.34094	UND	35.9816	38.9119	UND	UND
C1	UND	UND	UND	UND	UND	UND
D1	UND	UND	35.06582	UND	UND	UND
E1	28.09699	UND	33.37418	UND	0.0392	1.9608
F1	UND	UND	35.62026	UND	UND	UND
A2	29.94508	UND	38.24104	UND	0.0014	0.0693
B2	UND	UND	UND	UND	UND	UND
C2	34.41778	UND	37.77575	UND	0.0019	0.0954
D2	UND	UND	UND	UND	UND	UND
E2	33.11788	UND	36.16168	UND	0.0058	0.2891
F2	30.3889	UND	30.82519	UND	0.2258	11.291
A3	UND	UND	UND	UND	UND	UND
B3	UND	UND	36.78622	UND	UND	UND
C3	31.91066	UND	37.60868	UND	0.0021	0.107
D3	28.5434	UND	35.53677	UND	0.0089	0.444
E3	UND	UND	35.7951	UND	UND	UND
F3	39.25243	UND	37.4169	UND	0.0024	0.1221
A4	33.38193	UND	UND	UND	UND	UND
B4	UND	UND	39.90174	UND	UND	UND
C4	UND	UND	35.85213	UND	UND	UND
D4	UND	UND	UND	UND	UND	UND
E4	UND	UND	36.22931	UND	UND	UND
F4	UND	UND	UND	UND	UND	UND

Table 5 displays the success rates for each DNA extraction method and qPCR input volume. **Table 6** outlines the average DNA recovery (ng) and range of DNA recovery for each extraction method.

Table 5. Comparison of DNA recovery (qPCR) success based on DNA extraction method and input volume of DNA (2-µL versus a 1:1 dilution).

DNA extraction method	Human DNA recovered
Silica (2-µL)	0/9 samples
Organic (2-µL)	0/24 samples
Silica (1-µL)	6/24 samples
Organic (1-µL)	9/24 samples

Table 6. Comparison of average DNA recovery (ng) and range of DNA recovery (ng) based on DNA extraction method and input volume of DNA (2-µL versus a 1:1 dilution).

DNA extraction method	Average DNA recovery (ng)	Range of DNA recovery (ng)
Silica (2-µL)	0	0
Organic (2-µL)	0	0
Silica (1-µL)	0.0445	0.0151 - 0.1132
Organic (1-µL)	1.6929	0.0693 - 11.291

Although many of the soil samples yielded human DNA quantities within the range needed for autosomal STR genotyping (0.1ng – 1ng), the total quantities (ng) detected are likely underestimates of the total DNA present, as indicated by elevated IPC cycle threshold (C_T) values (which is commonly observed in inhibited samples).

CONCLUSIONS & FUTURE DIRECTIONS

This case study demonstrates proof-of-concept that it is possible to extract human nuclear (autosomal) DNA from soil collected underneath decomposing human remains. In this case, it was important to understand the underlying molecular biology, cellular components, and environmental variables when extracting, purifying, and quantifying the recovered human DNA. Understanding all variables at play in the burial environment enabled researchers to select an appropriate DNA extraction method and modify downstream protocols to maximize success.

Results from this research could serve as a primer for forensic investigation of suspected homicides and clandestine burial sites, both to confirm the previous presence of a decomposing body and/or to ultimately make a positive identification of the victim in the case. Since the remains used in this study were exhumed and genotyped for a private cemetery case, the autosomal STR profile is known for this female subject. Future testing will involve PCR amplification and genotyping of the 20 core CODIS loci for each sample in this study for which human DNA was detected – in order to verify that the human DNA recovered from the soil samples matches the endogenous DNA profile of the decedent. Additional studies focused on optimizing DNA extraction methods and enhancing the removal of humic acid (HA) and fulvic acid (FA) inhibitors should be explored.

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