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Studying the Role of Soil Factors on DNA Profiles of Human Remains

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Abstract: The generation of DNA profiles from skeletal remains is an important part of the identification process in both mass disaster and unidentified person cases. Since bones are the best biological materials remaining after exposure to soil factors and other environmental conditions and in cases where many years have passed since the of the individual,s death, the ability to purify large quantities of informative DNA from these hard tissues would be beneficial. **Methods:** Seventy femur male bone samples were collected from two different environmental mass graves (Al-Mahawiel mass grave) in Babil governorate and (Wadi Al-Salam mass grave) in Al-Najaf governorate in Iraq. The DNA from bone sample extracts was assessed by capillary electrophoresis and a standardized set of short tandem repeat (STR) loci were analyzed using Mutiplex 21 STR loci System to allow human identification by matching the profile of the dead individuals with their relatives. From these two different sites; soil samples were collected to analyze and study the different environmental factors and find a relation between the degree of preservation or degradation with factors like temperature, humidity, salinity, PH and texture of soil and find how these factors can affect on pattern of degradation of DNA profiles. **Results:** average of the percentage of the detected alleles in each locus in the two sites; was 87% for the Al-Mahawiel site and 71% for the Al-Najaf site. PH was 8.2 for the Al-Mahawiel site soil where as pH was 8.1 for Al-Najaf site soil. The Humidity in the clay soil of the Al-Mahawiel site was7.5% while it was 3.5% for the sandy soils in Al- Al-Najaf site. The total soluble salts were 1.9% in clay soil of the Al-Mahawiel site, while it was 24.1% in Al -Najaf sandy soil. **Conclusion**: clay soil, low salt concentration, PH slightly alkaline and low humidity all participated in increasing DNA preservation in the Al-Mahawiel site while the sand soil and high salt concentration participated in increasing DNA degradation in the Al -Najaf site.

Keywords: Skeletal remains, Mass grave, Locus drop out, DNA degradation.

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Introduction

Forensic anthropology utilizes the methods of physical anthropology to examine skeletal remains, especially in cases where the remains are in a state of decomposition or their identity is unknown. DNA fingerprinting is useful in determining relationships between individuals(1,2). It can establish paternity, confirm the parents of adopted children (3), resolve family disputes(4,5); and even settle inheritance cases. DNA can provide valuable information about family connections (6,7). Short tandem repeat (STR) loci are highly variable DNA markers composed of short, repeated sequences ranging from 1 to 6 base pairs in length. These STR loci are widely used in forensic DNA analysis

due to their high polymorphism (8,9). The Mass Graves Department in the Iraqi Medico-Legal directorate which was established in 2010 works to identify the missing individuals and it has developed its capacity in forensic anthropology and forensic genetics to recover and identify more individuals (10). In international law, the term "mass grave" is not officially defined, but it generally refers to a site where multiple human remains are buried. In Iraqi national law, a "mass grave" is defined as a land or location that contains the remains of more than one victim, who were buried or hidden. Identifying individuals from mass graves can be complicated due to different factors that affect the process (11). Mass grave identification is challenging due to decomposition, soil activity, and lack of witnesses. One limitation is the difficulty in obtaining a DNA profile from bones undergoing extensive diagenesis. In some cases, the destructive burial environment can prevent the production of a usable DNA profile (12). A variety of environmental factors can act to create differential preservation of the DNA in human remains samples (13) like: temperature (14), humidity(15), salinity, PH (16); and texture of soil (17).

Materials and methods

Seventy femur bone samples were taken from remains of adult males' bodies stored in deep freeze (-80 C) in Iraqi Medico-Legal directorate lab, collected in 2011 after being buried for 20 years since they were killed by Saddam Hussein's regime in 1991. These samples were recovered from two different environmental, soil type mass graves.

Thirty-five samples were taken from the Al-Mahawiel mass grave in Babil Governorate and 35 samples from the Al-Najaf mass grave in Al-Najaf Governorate. Soil samples were taken from each mass grave, analyzed and studied at the National Center for Laboratories and Structural Research in Baghdad, which has the national accreditation ISO/IEC.TL009 according to standard specifications. The present study was carried out in the Medico legal directorate in Baghdad Iraq. The skeletal samples displayed high levels of degradation so they were impossible to get them identified by personal effects and other secondary methods of identification. The degradation processes rendered all the bodies in a deformed shape.

From these two different sites, soil samples were collected to analyze and study the soil environmental factors that affect the quality of DNA profiles, the intensity of preservation or degradation of the DNA of these samples and the pattern of the profiles.

Many steps were performed according to the protocol used in the DNA Laboratory for the identification of mass graves victims in Medico-Legal directorate to extract DNA from bone samples.

Firstly, steps of preparing samples were performed including weighting between 0.5 and 1.0 g of bone powder taken from washed, purified and grinding bone. Secondly, extraction process was done according to the following steps, involving using digestion buffer and Proteinase K to precipitate any bone debris by centrifugation and taking the supernatant then Amicon Ultra was

used to recover DNA by washing with ethanol, and any residual ethanol was removed by centrifuging samples for 1 min at 13,000 rpm to elute the DNA.

Quantification of extracted DNA was performed by using 7500HID Real Time PCR from Applied Biosystems and Quantifiler Human DNA quantification kit. PCR protochol follower the Applied Biosystems 9700 thermocycler using the standard manufacturer's recommended conditions (Figure 1). Mutiplex 21 STR loci System was used for human identification applications including forensic analysis, relationship testing and research. The system allows co-amplification and four-color fluorescent detection of 21 loci, described in (Table 1) and illustrated in (Figure 2).

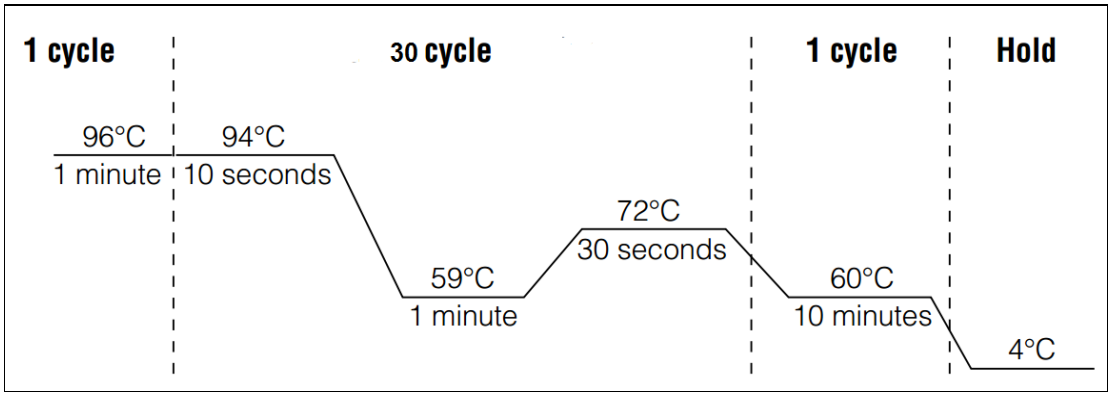


Figure (1): The thermal cycling protocol for the GeneAmp® PCR System 9700 thermal cycler for extracted DNA.

Table (1): Four-color fluorescent detection of 21 loci.

No.	Loci	Fluorescent Dye
1	D3S1358, D1S1656, D6S1043, D13S317, Penta E	Blue
2	D16S539, D18S51, D2S1338, CSF1PO, Penta D	Green
3	TH01, vWA, D21S11, D7S820, D5S818, TPOX	Yellow
4	D8S1179, D12S39, D19S433, FGA	Red

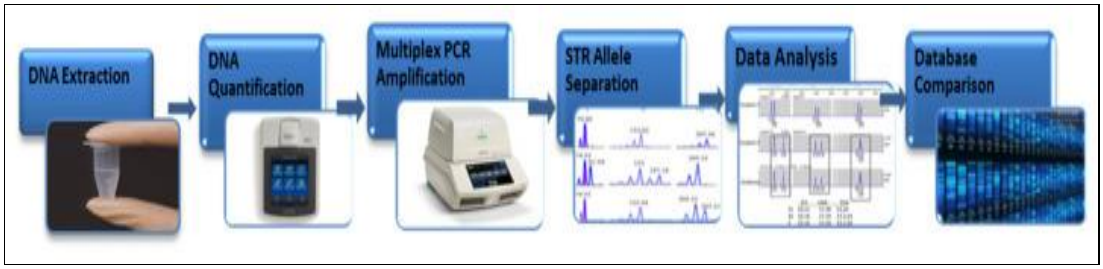


Figure (2): Workflow of STR typing.

Capillary electrophoresis was performed using a 3500XL Genetic Analyzer (Applied Biosystems, Foster City, USA) in accordance by the manufacturer's instructions; ILS-500 (Promega) was used as an internal size standard. Genotyping was performed using GeneMapper ID-X v1.4 software (Applied Biosystems). Alleles were designated according to the published nomenclature; off ladders were called if they fell into a virtual bin as long as the profile as a whole was judged to be of good quality, alleles that were not in a

bin or virtual bin were re-run at least once before designation (18).

Once profiles are obtained, statistical calculations are conducted on the data to evaluate which type of soil and environmental factors were more effective in causing degradation to the DNA of bone samples. After calculation of the number of profiles that succeeded to detect alleles in the mentioned locus in all the 35 profiles in each one of the mass graves sites, the percentage of each locus that succeeded in detect alleles can be calculated by application the following equation; the results are shown in (Table 2).

$$\frac{\text{Number of profiles succeeded to detect alleles in the mentioned locus}}{35} * 100\%$$

Results and discussion

Case history

International organizations estimate that more than 400,000 Iraqis were killed by regime security forces during Saddam's rule, but some estimates are as high as 1 million. At least 270 mass graves have been reported by Iraqis and U.S. (19). The remains femur bone samples were taken from remains of adult males' bodies from two different sites of mass graves to recover their DNA profiles and make a comparison between the degrees of degradation between the two sites which reflects the effect of soil factors on these profiles.

DNA profiling

Bone samples that were taken from remains were received by the DNA lab. Collection of samples, DNA Extraction, quantification, amplification and capillary electrophoresis, all these processes were done according to the standard operating procedure of DNA Lab. for the identification of missing persons in MLD in Bagdad Iraq

After getting DNA profiles for these bone samples, the tests of the soil samples, which included measuring soil salinity, acidity, soil texture and temperature, were compared with the pattern of the genetic fingerprints for each mass grave. After categorizing these results of the profiles into 3 groups; complete (or missing one or two loci), partial (missing more than 3 loci) and failed profiles. We conclude that the data from Al-Mahawiel were 29 complete (or missing one or two loci) DNA profiles obtained from different bone remains samples. Three Partial (missing more than 3 loci) DNA profiles were obtained from different bone samples. Three bone samples failed to obtain any DNA profiles because they were highly degraded. The data from Al-Najaf mass graves profiles were: Twenty-Three complete (or missing one or two loci) DNA profiles obtained from different bone samples. Five partial (missing more than 3 locus) DNA profiles were obtained from different bone samples. Seven bone samples failed to obtain any DNA profiles because they were highly degraded. These results are represented in (Figure 3).

Statistical analysis

Once profiles are obtained, statistical calculations are conducted on the data to evaluate which type of soil

and environmental factors were more effective in causing degradation to the DNA of bone samples. The number of profiles that succeed in detecting alleles in each mentioned locus in all the 35 profiles in each mass graves site was calculated as shown in (Table 2), and the percentage of each locus that succeeded in detecting alleles also were calculated as shown in (Table 3).

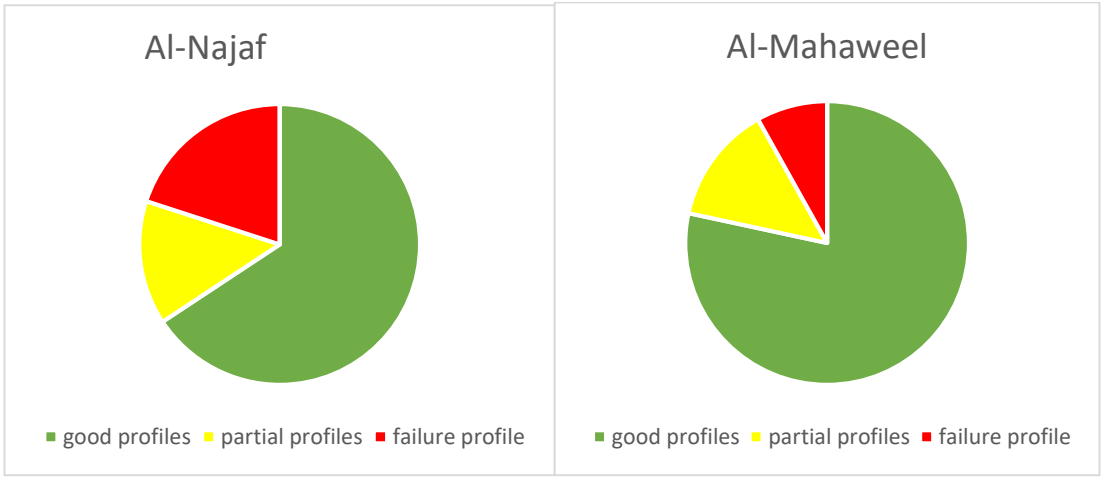


Figure (3): Represent the quality of DNA profiles that were recovered from bone samples from the two sites.

Table (2): Number of profiles that succeed to detect alleles in each mentioned locus in Al-Mahaweel and Al-Najaf massgraves.

Locus	Number of DNA profiles that succeed to detect alleles of the mentioned locus in Al-mahaweel mass grave	Number of DNA profiles that succeed to detect alleles of the mentioned locus in Al-Najaf mass grave
AMEL	Detected in 32 profiles	Appears in 28 profiles
D3S1358	Detected in 32 profiles	Appears in 28 profiles
D1S1656	Detected in 31 profiles	Appears in 26 profiles
D6S1043	Detected in 31 profiles	Appears in 25 profiles
D13S317	Detected in 28 profiles	Appears in 24 profiles
Penta E	Detected in 27 profiles	Appears in 21 profiles
D16S539	Detected in 32 profiles	Appears in 27 profiles
D18S51	Detected in 32 profiles	Appears in 27 profiles
D2S1338	Detected in 32 profiles	Appears in 27 profiles
CSF1PO	Detected in 28 profiles	Appears in 22 profiles
Penta D	Detected in 32 profiles	Appears in 24 profiles
TH01	Detected in 32 profiles	Appears in 28 profiles
VWA	Detected in 32 profiles	Appears in 27 profiles
D21S11	Detected in 31 profiles	Appears in 25 profiles
D7S820	Detected in 31 profiles	Appears in 24 profiles
D5S818	Detected in 28 profiles	Appears in 23 profiles
TPOX	Detected in 27 profiles	Appears in 20 profiles
D8S1179	Detected in 32 profiles	Appears in 26 profiles
D12S391	Detected in 32 profiles	Appears in 27 profiles
D19S433	Detected in 31 profiles	Appears in 26 profiles
FGA	Detected in 31 profiles	Appears in 26 profiles

Table (3): Percentage of alleles detection in each mentioned locus in both massgraves.

Locus	Percentage of the mentioned locus that succeeded to detect alleles in Al-Mahaweel site samples	Percentage of the mentioned locus that succeeded to detect alleles in Al-Najaf massgraves samples
AMEL	91%	80%
D3S1358	91%	80%
D1S1656	88%	74%
D6S1043	88%	71%
D13S317	80%	68%
Penta E	77%	60%
D16S539	91%	77%
D18S51	91%	77%
D2S1338	91%	77%
CSF1PO	80%	62%
Penta D	91%	68%
TH01	91%	80%
VWA	91%	77%
D21S11	88%	71%
D7S820	88%	68%
D5S818	80%	65%
TPOX	77%	57%
D8S1179	91%	74%
D12S391	91%	77%
D19S433	88%	74%
FGA	88%	74%

A Comparison of Percentage of alleles detection of each locus between Al-Mahawiel mass grave samples and Al-Najaf mass grave samples were studied by representing these results in (Figure 4).

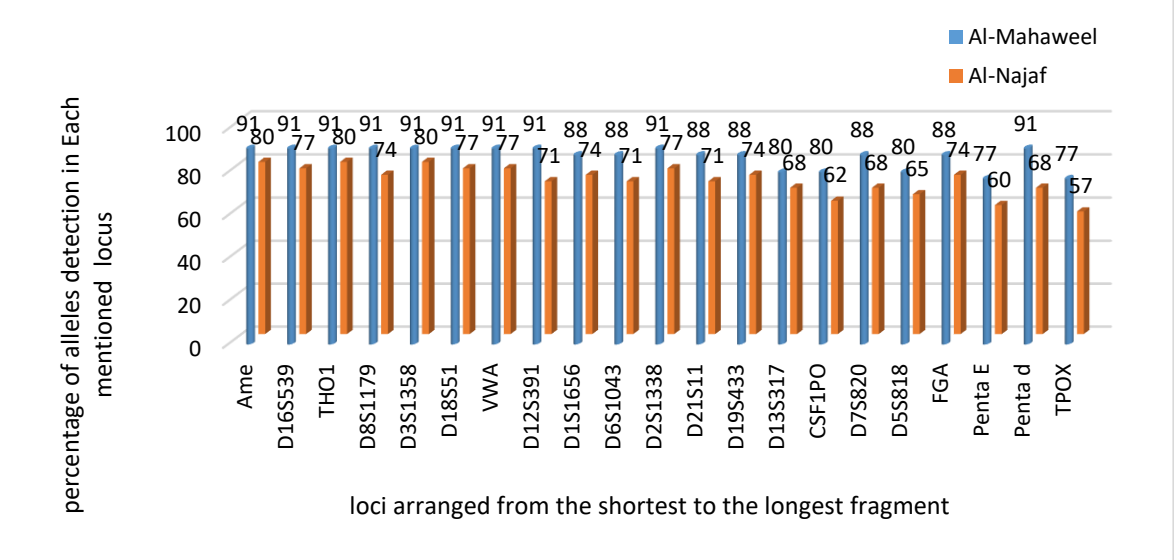


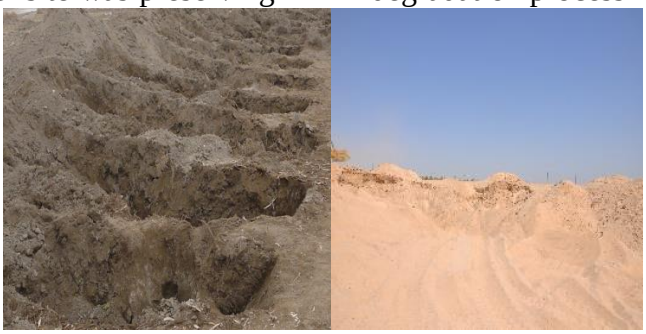
Figure (4): A Comparison of the Percentage of alleles detection of each locus between Al-Mahaweel mass grave samples and Al-Najaf mass grave samples.

This figure gave a simple, but clear understanding to the quality of profiles in each site reflecting the degradation and preservation degree and the effect of soil environmental factors on the percentage of detection of alleles in each mentioned locus. After calculation the average of the percentage of the recovered alleles in each locus in the two sites; was 87% for the Al-Mahaweel site and 71% for Al-Najaf site. This illustrates that the

environmental factors in the A-Mahaweel site give more preserving for the DNA, while the environmental factors in the Al-Najaf site were more degrading for DNA.

Soil test results

For the Al-Mahaweel site, soil test results show pH was 8.2 which is considered slightly alkaline so it gave good preservation for DNA. In the Al-Najaf site soil pH which was 8.1. DNA is less prone to damage in neutral or near- neutral environments (15). The Humidity in soil of the Al-Mahawiel site was 7.5% which is low so it preserves the DNA because low humidity does not support the growth microorganisms that play an important role in the degradation of bone material, also this low humidity could be enough to lower the high temperature during hot summer in Iraq which reach to 53 C, this cooling effect might increase the preservation of the DNA. The total soluble salts were 1.9% which is low compared with the Al- Al-Najaf site, which was 24.1% ,this gave more preserving to DNA because soil high in salt content can be highly destructive to bone (16). Soil texture which was clay in the Al-Mahaweel site was preserving factor because clay soils that retain moisture as a result of their small particle size will generally retard decomposition rates to a greater extent than more permeable more dried (humidity 3.5%) sandy soils in the Al-Najaf site (16) as the soil permeability affects water permeability and air exchange within the soil, this occurs because the concentration of oxygen in water is much lower than in air, and oxygen diffusion through water is very slow (17).Soil texture for both sites shown in (Figure 5). A higher likelihood of the longer fragments being degraded compared to the shorter fragments. A probable explanation for this is that the longer the repeat sequence the higher the probability of a breakage between the primer binding sites. Consequently, signals from the longest alleles are frequently missing. The phenomenon is called allelic drop-out if only one of the two alleles in a locus is missing. If no result is observed in a locus, the phenomenon is called locus drop-out (18). Further studies are planned to get more information about the types of micro-organisms that contributed to the degradation process in each site.



(a) **(b)**

Figure (5): a) Al-mahaweel mass graves site, b) Al-Najaf massgrave soil

Conclusion

This study is the first of its kind in Iraq. After studying the assessment of the factors of soil type and another environmental factor that affect DNA degradation or preservation and profile of STR pattern. It was found that clay soil, low salt concentration, PH slightly alkaline and low humidity all participate in DNA preservation while the sand soil

and high salt concentration participate in DNA degradation. Predicting the effect of these factors on the quality of the DNA profiles can be help find better solutions for many complications that may face the process of analyzing samples and finding sufficient informative profiles. In addition, studying and explaining the effect of these factors on the quality of DNA profiles will give scientific, reasonable and satisfactory excuses to the victims' families to make them aware of why the DNA profiles of their sons are still not obtained in some cases.

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