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Extraction of DNA from Bloodstained Fabric Samples Buried in Different Types of Soil

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Bloodstained clothing provides important evidence in murder crime scenes as the DNA on clothing can aid in identifying both victims and possible suspects. This is because some homicide cases involve the victims being fully or partially buried in their bloodstained clothes or found with their bloody clothes buried nearby. The aim of this research is to study the persistence of DNA extracted from ten types of bloodstained fabric samples previously buried in four types of soil. The ten types of fabrics used for this study were satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette. The soil types used in this study were sandy, silt loam, loamy sand and clay loam soil. The DNA extractions from bloodstained fabric samples were made by using NEXPreo Blood DNA Mini Kit. The concentration of DNA from the extracted samples were analysed by using Eppendorf BioPhotometer Plus. The DNA in linen fabric degraded fastest in all four types of soil as it showed no presence of DNA on day 15 whilst cotton has the highest retained DNA concentration in all soil samples. Sandy soil showed the highest DNA concentration retention for all type of fabrics except linen while clay loam soil showed the most rapid DNA concentration degradation for all types of fabrics. Cotton fabric was able to retain the pure DNA up to day 10 while lycra fabric retained pure DNA only up to day 5 in silt loam soil. Both lycra and georgette fabrics managed to preserve the pure DNA until day 5 in sandy soil. In comparison, only georgette fabric retained pure DNA until day 5 in loamy sand soil. Finally, all types of fabrics buried in clay loam soil were not able to even preserve the pure DNA to day 5. This research indicates that it is critical to locate and retrieve any buried blood-based evidence as soon as possible to prevent further evidence loss due to the varying characteristics of the different types of fabrics and soils which could encourage microbial development as well as provide other ways to degrade DNA.

Keywords: Bloodstained fabrics; DNA; clay loam soil; silt loam soil; sandy soil; loamy sand soil

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Bloodstained fabrics play a vital role in DNA analysis crime investigation [1]. Environmental conditions such as heat, moisture and microorganisms can lead to the deterioration of any biological trace. This includes rainfall and uncontrollable conditions that can affect the characteristics of the biological evidence, mainly DNA and bloodstains. The key point in blood-based evidence is that blood can degrade if suspects were to dispose the bloodied clothing evidence by burying them [2]. The particle size of soil could affect the decomposition process of blood on fabrics. Different textures of soils for example, fine- textured soil have lower rate of gas diffusion than the coarse-textured soil, therefore oxygen-CO_2 exchange might not be sufficient to produce aerobic microbes and as the result decomposition can be retarded [3]. Microorganisms have high capability to proliferate in soil after the samples have been collected which gradually affect the DNA profiles. Additionally, some fungal species can withstand colder temperatures which could be a crucial precaution for long-term storage where DNA extraction needed to be carried out shortly after it has been collected from the soil [4]. Decomposition process in a buried environment has been a research investigation agenda for over a decade, before forensic taphonomy was introduced [5]. Understanding the persistence of DNA when extracted from different types of bloodstained fabrics buried in different types of soil is crucial as the burial environment can alter the decomposition of the DNA. Factors that can affect the DNA decomposition within a burial site involve the burial depth, clothing type and the conditions of the soil itself. Forensic soil analysis is a significant study that spans several fields of research because soils are unique and can be characterized using several criteria, including texture, mineralogy, consistency, particle size, pH and colour [4]. Therefore, a clear need exists for dedicated studies on the persistence of human DNA in soil, as the mere presence of human DNA in the soil can provide useful information in certain circumstances. Most of the research investigation relating to DNA extraction involving soils and fabrics were conducted overseas

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with different climates, environmental factors as well as physical soil conditions compared to Malaysia. All these variable factors can affect the DNA extraction results. It is therefore crucial to conduct, increase and expand blood analysis forensic studies relating to fabrics and soils as it has become relevant due to the manner of most crime committed nowadays in Malaysia. This current study involves analyzing degrading human blood samples from ten different fabrics which were buried in four different soils up to twenty days. Data from this study could contribute significantly to the Malaysian forensic databank relating to DNA persistence on different types of bloodstained fabric samples buried in different types of soils.

EXPERIMENTAL

Sample Preparation

Ten types of fabrics; satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette were obtained for this study. The fabrics were categorised into natural fabrics (cotton, satin, chiffon, linen) and synthetic fabrics (crepe, argenti, valentro, lycra, georgette, and polyester). The fabrics were cut into square pieces of size 14cm by 14cm. Each fabric sample was prepared in four replicates for four different types of soils. A pool of human blood source was obtained for this study and about 20ml was poured on the 40 pre-cut fabrics samples to ensure the fabrics were fully covered with blood. The fabric samples were then left drying at room temperature (26°C to 29°C) for 72 hours. After the drying process, 2cm by 2cm piece from each fabric replicates were cut using sterilized scissors to be used as standards. Then the fabrics containing the dried blood were stored in labelled paper envelope separately for each replicate and were preserved in a freezer with temperature of -20 °C. A total of 40 fabrics samples were buried approximately 12cm beneath the surface of each soil type. The sample preparations were based on a previous study [6] with minor modifications.

Sample Collection

Sample collections were performed on the 5th, 10th, 15th, and 20th day. During each sampling time, 2cm by 2cm size cloths were cut off the bloodstained fabrics from each soil using sterilized scissors. The collected samples were air-dried and kept into labelled brown envelopes before being preserved at -20°C as recommended by Hara et al. [7] to prevent DNA degradation during long-term storage. The field site used for this study is located within Universiti Teknologi MARA campus in Shah Alam, Selangor, Malaysia. Four types of soil, clay loam soil, silt loam soil, sandy soil, and loamy sand soil were identified based on the soil texture tests. Parameters of soil moisture content, bulk density, porosity, pH and temperature as mentioned in study [8] were conducted using instructions given in the Laboratory Workbook for Method of Analysis for Soil [9].

DNA Extraction

DNA extraction of the bloodstained samples was performed using NEXprep^{TM} NexK-3100 Genomic Mini Kit (Blood DNA Mini Kit, 100 prep). All the solutions preparations and extraction steps were performed according to the manufacturer's instructions.

DNA Quantification

The concentration of DNA in each of the samples was measured using Eppendorf BioPhotometer Plus. About 5 μL final extractant was pipetted into a 1.5 mL tube and diluted with 55 μL of distilled water, then placed in a Uvette cuvette. The Uvette cuvette was placed on the BioPhotometer Plus and the concentration of DNA for the sample was noted. The wavelengths used for these measurements were 260 nm for nucleic acids detection and 280 nm for proteins and phenol. The ratio of $A260/A280$ represents the purity of the DNA contained in the fabric sample from a range of 1.800 to 2.000, and if the value is less than 1.800, the DNA in the fabric sample is not pure [10].

Soil Moisture Content

Gravimetric or drying method is a direct method of determining the moisture content by drying the soil sample and measuring the difference between the original and dried sample weight. The excavation method involves digging out one scoop of soil. Then the soil sample is cleaned from organic and mineral debris and weighed in an aluminium boat. This soil sample is dried at 105 °C in the oven overnight until all the moisture is evaporated. The following day, the soil sample is cooled in a desiccator for 30 minutes and re-weighed. The resulting difference in mass represents the water that has evaporated. This mass is then incorporated into a formula to calculate the soil moisture in weight percentages (% mass). This experiment was conducted in triplicate for each soil type.

Soil Bulk Density

The excavation method involved digging out one scoop of soil sample. The sample were spread over a paper to remove the debris and rocks. The debris free soil sample was weighed, and oven dried at 105 °C over-night. The next day the soil sample was cooled in a desiccator for 30 minutes and later sieved and screened over a large sheet of paper. The dried soil sample was weighed. The isolated rocks and other large debris were immersed in a 100 mL graduated cylinder filled with only 30 mL of water. The displaced water volume representing the volume of the debris was recorded for further calculations. This procedure was conducted in triplicate for each soil types.

Table 1(a). Average soil moisture content in four different types of soil.

Type of soil	Moisture content (%)
Sandy soil	20.86
Loamy sand soil	22.26
Silt loam soil	23.53
Clay loam soil	25.02

Table 1(b). Average bulk density for each soil.

Type of soil	Bulk density (g/cm^3)
Sandy soil	2.000
Loamy sand soil	1.585
Silt loam soil	1.311
Clay loam soil	1.068

Table 1(c). Average soil porosity for each soil.

Type of soil	Soil porosity (%)
Sandy soil	25.0
Loamy sand soil	35.1
Silt loam soil	42.3
Clay loam soil	47.9

Table 1(d). Average soil pH value for 4 different types of soils.

Type of soil	pH
Sandy soil	6.68
Loamy sand soil	5.96
Silt loam soil	4.34
Clay loam soil	3.67

Table 1(e). Average temperature for each soil and its surrounding.

Day	Type of soil							
	Sandy soil		Loamy sand soil		Silt loam soil		Clay loam soil	
	Soil	Surrounding	Soil	Surrounding	Soil	Surrounding	Soil	Surrounding
5	26.6°C	33.3°C	27.4°C	32.1°C	28.6°C	33.2°C	37.8°C	31.9°C
10	29.3°C	25.9°C	26.2°C	28.4°C	26.9°C	31.2°C	35.8°C	28.3°C
15	25.9°C	32.9°C	29.1°C	29.8°C	28.4°C	30.9°C	37.2°C	31.5°C
20	25.9°C	31.7°C	28.9°C	32.8°C	26.9°C	31.9°C	37.5°C	30.9°C

Porosity of Soil

This soil characteristic was investigated by filling a cup to the 100 mL level with the tested soil. Then 100 mL water was added from the graduated cylinder until the water level reached the top of the soil in the cup. The amount of water left in the graduated cylinder was noted and subtracted from the original 100 mL. This volume was used to calculate the pore space. This procedure was performed in triplicate for each soil type.

Soil pH

The pH meter was calibrated with known buffer solutions of pH 4.0 and 9.2. 20 g of soil was weighed and transferred into a 100 mL beaker. 40 mL distilled water was added and stirred well with a glass rod. This mixture was allowed to stand for 30 mins. The pH electrode was immersed to obtain pH of the mixture. This procedure was performed in triplicate for each soil type.

Temperature of Soils & Surroundings

The temperature of the soils and the surroundings were taken and recorded daily for 20 days using a digital and portable thermometer (Extech). The readings were recorded in triplicate daily for each soil type and the surroundings.

RESULTS AND DISCUSSION

Parameters for Soil Samples

The bloodstained fabric samples were buried approximately 12cm beneath the surface of the soil for 20 consecutive days. Soil moisture content reflects the volume of water in the soil, which depends on the soil type. Soil moisture for all the soil samples were calculated using % w/w formula. According to Table 1(a), the highest moisture content was from clay loam soil, followed by silt loam soil, loamy sand soil and the lowest was from sandy soil. Clay loam soil has the highest moisture content which could decrease the DNA concentration on the blood-stained evidence.

Bulk density describes the soil compaction, and it is an important parameter to soil health. Table 1(b) shows sandy soil has the highest soil bulk density value, followed by loamy sand soil, silt loam soil and finally clay loam soil. This shows that soils with high porosity which are loose and have the low bulk density such as clay loam soil help in good root growth of plants. On the other hand, sandy soil with higher bulk density have greater compaction will reduce and limit the root growth.

Soil porosity is described as empty spaces between the soil particles known as pore spaces which are engaged by air and water. Table 1(c) shows clay loam soil has the highest soil porosity with more micro pores compared to the other soils. In addition, clay soils are more susceptible to water logging which can adversely affect root respiration and microbial activity. Sandy soils also have lower moisture content when compared to clay loam soil, which is directly related to the porosity of the soil.

Soil pH plays an important role in DNA adsorption to clay minerals, as pH affects the electro-static property of both the clay mineral surface and the DNA phosphate. The electrostatic state of the clay mineral surface is dependent on the pH since there is no net charge on the clay surface. Soil dispersion is pH-dependent, and a soil subjected to a buffer with a high pH will result in better dispersion and more efficient DNA recovery [4]. Studies had shown an increase in DNA yield with an increase in extraction buffer pH, with an optimum of pH 9 [11] and an increase in DNA adsorption by soils with low pH value [12,13]. As shown in Table 1(d), clay loam soil has the lowest pH value and is the most acidic soil. Acidic soil condition accelerates the hydrolysis of nucleic acid, resulting in higher DNA degradation rate and sandy soils with the higher pH shows lower DNA degradation rate [2]. This point correlates well with the findings from this study, that showed higher DNA concentration for bloodstained fabrics buried in sandy soil compared to clay loam soil (Table 2).

Both DNA degradation and survival depends on temperature as it plays an important function. According to Table 1(e), it indicated that for all the tested days, clay loam soil showed the highest average temperature whilst the lowest average temperature was shown by sandy soil. As temperature increases, the DNA degradation increases with damages rapidly accumulating with time. In addition, DNA degradation also depends on the presence of water. High quantity of water in the air and elevated temperature could expose the fabric to the growth of biological materials and microorganisms such as bacteria and fungi resulting in rapid decay of DNA [14].

Overall Comparison of DNA Concentrations in All Fabrics

Based on Table 2, it can be concluded that the concentration of DNA for the 10 types of fabrics gradually declined from day 0 until day 20 for all types of soil. Most of the DNA concentration for 10 fabrics showed a negative value on day 20, which indicated that there was no presence of DNA except for linen, showed a negative value for the DNA concentration starting on day 15 for sandy soil and silt loam soil. Linen and georgette showed negative values of DNA concentration on day 15 for loamy sand soil, while all other fabrics showed negative values starting on day 15 for clay loam soil. This study also showed that all types of fabrics buried in clay loam soil have the lowest DNA concentration especially on day 15 compared to other types of soils.

Table 2. Concentration of DNA retained from bloodstained fabrics buried in different types of soil for 20 days.

Type of fabric	Average concentration of DNA in sandy soil (ng/µL)					Average concentration of DNA silt loam soil (ng/µL)				
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 0	Day 5	Day 10	Day 15	Day 20
Polyester	405.8	312.1	224.7	96.3	-23.4	512.3	357.8	150.9	36.2	-52.3
Satin	385.7	123.4	68.3	23.9	-15.1	404.0	372.6	134.0	25.9	-31.9
Chiffon	527.1	479.4	137.0	42.3	-6.07	391.0	274.6	45.0	1.3	-38.7
Cotton	623.9	488.6	258.2	104.1	-1.02	530.7	445.6	338.5	69.3	-23.3
Linen	125.4	111.9	78.5	-5.53	-23.9	231.4	202.6	67.4	-56.1	-85.9
Argenti	171.1	164.8	72.8	37.2	-6.03	444.1	231.4	86.5	23.4	-15.1
Valentro	300.8	200.5	101.5	65.7	-3.02	493.7	293.3	137.9	12.7	-39.5
Crepe	328.9	198.5	101.5	25.4	-11.1	359.6	258.4	72.1	1.74	-31.6
Lycra	448.1	258.2	149.3	60.6	-1.63	211.9	111.1	74.2	12.1	-47.2
Georgette	369.6	78.2	60.1	23.8	-35.3	125.4	78.2	60.1	23.3	-45.3
Type of fabric	Average concentration of DNA in loamy sand soil (ng/µL)					Average concentration of DNA in clay loam soil (ng/µL)				
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 0	Day 5	Day 10	Day 15	Day 20
Polyester	459.1	230.9	103.9	53.2	-22.3	261.6	121.8	78.6	-10.2	-62.3
Satin	479.2	356.9	133.7	13.1	-20.1	328.3	132.6	34.6	-51.5	-69.0
Chiffon	342.3	225.4	58.8	33.3	-42.7	309.8	1.97.0	42.3	-26.1	-26.8
Cotton	518.4	354.8	101.8	56.1	-13.3	390.7	142.5	85.6	-6.1	-11.2
Linen	170.0	117.8	27.4	-56.1	-93.9	361.6	210.1	74.8	-61.0	-83.9
Argenti	277.1	226.2	117.2	26.4	-35.2	269.8	116.9	49.2	-30.2	-78.6
Valentro	182.3	144.9	91.3	23.4	-47.5	301.3	184.9	75.9	-13.4	-25.5
Crepe	353.1	84.9	44.6	13.4	-56.5	223.1	117.9	35.9	-34.9	-59.5
Lycra	239.5	200.6	72.3	27.5	-17.2	291.2	109.6	68.6	-24.8	-62.3
Georgette	217.3	97.9	25.8	-14.1	-24.7	343.4	101.9	57.7	-25.1	-34.9

Table 3 reveals the DNA purity for standard sample (Day 0) from 10 fabrics was pure before being buried under the soil. However, only lycra and georgette fabrics buried in sandy soil, cotton and lycra fabrics buried in silt loam soil and georgette fabric buried in loamy sand soil showed ratio more than 1.800 indicating the extracted DNA were still pure on day 5. Nevertheless, the purity of DNA for all types of fabrics buried in different types of soils showed the ratio of purity lower than 1.800. A lower ratio of DNA purity indicated that the extracted DNA had been contaminated. According to [10], the ratio for DNA purity must be 1.800 and above. Cotton fabric was able to retain pure DNA until day 10 when buried in silt loam soil.

Environmental conditions such as heat, moisture and microorganisms lead to the deterioration of biological evidence such as blood. This includes rainfall and uncontrollable conditions that affect the characteristic of the biological evidence, mainly DNA in bloodstains [2]. Rainfalls give greater impact to soil moisture, pH, organic carbon that can lead to a change in soil microbial community structure. Active microbial growth can cause rapid degradation of DNA, thus altering the final result [15]. Heavy metal pollution caused by rainfall would increase DNA damage which can alter the outcome of DNA extraction [16].

Soil mineralogy and composition can influence DNA preservation in soil. Clay, silt, and sand have each demonstrated different degrees of DNA binding capabilities, with clay binding DNA the strongest, followed by silt, and then sand. Depending on the clay composition, soil conditions may or may not be conducive to DNA adsorption to the soil matrix [8]. Soil microbes are able to remain active when nutrients are sufficient. Lack of nutrient supply to the soil microbial biomass in dry soils will effectively reduce cadaver decomposition process [17]. A wide range of factors including soil type, pH, temperature, humidity, microbial activity play roles in the cadaver decomposition process. The decrease of the grave soil pH accelerated the decomposition rate. Sand and silt type soils were

able to increase the decomposition process with more moisture and higher temperate environment [3]. Hence, the type of soil is one important factor that affects the decomposition of any organic material in soil. Figure 1 shows the effect of decomposition on the linen fabric sample when buried in clay loam soil for 20 days.

Table 3. Purity of DNA retained from bloodstained fabrics buried in different types of soil for 20 days.

Type of fabric	Purity of DNA in sandy soil (ng/µL)					Purity of DNA silt loam soil (ng/µL)				
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 0	Day 5	Day 10	Day 15	Day 20
Polyester	2.422	1.765	1.114	0.897	0.671	1.891	1.187	0.847	0.698	0.612
Satin	1.914	1.762	1.169	0.785	0.433	2.231	1.741	1.232	1.031	0.635
Chiffon	1.813	1.221	1.126	0.818	0.806	1.893	1.754	1.364	0.873	0.534
Cotton	1.877	1.532	1.119	1.001	0.806	2.774	2.141	1.893	1.332	1.213
Linen	1.981	1.662	1.241	0.849	0.311	1.993	1.762	0.928	0.753	0.129
Argenti	2.451	1.671	1.245	1.142	0.712	1.987	1.672	1.223	0.872	0.645
Valentro	1.955	1.567	1.068	0.956	0.651	1.811	1.789	1.224	0.848	0.622
Crepe	1.954	1.771	1.508	1.113	0.873	2.563	1.763	1.222	1.005	0.746
Lycra	2.340	1.873	1.654	1.223	0.879	2.140	1.983	1.517	1.114	0.078
Georgette	1.976	1.841	1.661	1.321	0.893	2.642	1.677	1.534	1.295	0.651
Type of fabric	Purity of DNA in loamy sand soil (ng/µL)					Purity of DNA in clay loam soil (ng/µL)				
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 0	Day 5	Day 10	Day 15	Day 20
Polyester	1.893	1.650	1.267	0.997	0.659	1.975	1.254	1.142	0.896	0.659
Satin	2.121	1.693	1.062	0.972	0.443	1.878	1.667	0.929	0.815	0.132
Chiffon	1.859	1.341	1.274	0.857	0.115	1.818	1.297	1.269	1.113	0.992
Cotton	1.899	1.334	0.979	0.445	0.047	2.375	1.798	1.065	1.015	0.953
Linen	2.125	1.770	1.735	1.071	0.751	2.228	1.665	1.171	1.071	0.752
Argenti	3.782	1.551	1.117	0.813	0.312	1.850	1.350	1.026	0.817	0.633
Valentro	1.893	1.593	0.895	0.167	0.069	2.430	1.729	1.548	0.974	0.668
Crepe	2.773	1.664	0.457	0.162	0.157	2.103	1.761	1.431	0.931	0.561
Lycra	2.604	1.233	1.070	1.001	0.926	2.893	1.727	1.137	1.065	0.595
Georgette	2.345	2.000	1.375	1.109	0.789	2.001	1.493	1.229	0.983	0.594

* If the value is less than 1.8, the extracted DNA from the fabric samples is not pure

Figure 1. Condition of linen fabric before and after being buried in clay loam soil for 20 days.

CONCLUSION

In conclusion, DNA extraction from different bloodstained fabrics buried under different types of soils are influenced by both environmental and chemical factors. In this preliminary research, DNA in natural linen fabric degrades fastest in all 4 types of soils as it shows no presence of DNA on day 15 whilst cotton had the highest DNA concentration retained in all soil samples. Cotton is able to retain pure DNA for the longest until day 10 when buried in silt loam soil. On the other hand, sandy soil showed the highest DNA concentration retained for all types of fabric for the period of 20 days while clay loam soil shows the most rapid DNA degradation for all types of fabric. This study indicates that the type of soils, surrounding environmental conditions as well as type of fabrics all play important roles in the DNA extraction process for bloodstained buried evidence.

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