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DNA Extraction from Bloodstained Fabric Samples Immersed in Different Types of Water

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Biological fluid especially human blood is a type of evidence that plays a major role in criminal investigations. However, there are challenges in performing blood DNA analysis due to environmental factors and criminal acts that affect DNA stability. This usually happens when dead bodies of victims are subjected to water or land burial. Samples found in such crime scenes often lack sufficient evidence due to DNA degradation or contamination. The aim of this research is to study the persistence of DNA extracted from different types of bloodstained fabric samples immersed in different types of water. Ten types of fabrics used for this study were satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette. The four water samples were from a lake, a river, the sea, and tap water. The extraction of DNA from bloodstained fabric samples were conducted using NEXPreo Blood DNA Mini Kit. The concentration of extracted DNA were analysed with Eppendorf BioPhotometer Plus. Fabrics immersed in tap water had the highest DNA concentration retention followed by lake water, river water and sea water. Among the ten types of fabrics, synthetic fabrics have better retention of DNA concentrations compared to natural fabrics. The concentrations of DNA from all type of fabrics drastically decreased to almost half of the DNA concentration within 24 hours of immersion for all types of water. This study also showed that underwater DNA loss and DNA degradation was very high within the first 48 hours compared to 72 hours reported in previous studies. Future studies are recommended to reduce the time of immersed fabrics collection to hourly instead of daily so that it could provide more valuable information for crime investigations.

Keywords: Fabrics; DNA; tap water; lake water; river water; sea water

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Biological evidence such as bloodstains found at crime scene is useful scientific evidence that can provide vital information about victims, criminals or weapons found at crime scenes [1,2]. Biological evidence is highly related to DNA analysis therefore, detection and recovery of biological liquids is important to utilize the DNA evidence. Interpretation of DNA evidence are highly influenced by hostile conditions at the crime scene [3]. There are challenges in performing DNA analysis due to environmental factors which play a major role in DNA stability. This is particularly because dead bodies or parts of them are usually found carbonized, submersed or buried at crime scenes are insufficient for DNA analysis due to DNA degradation and contamination [4,5]. The rate of DNA degradation depends on light conditions, water content, temperature and contamination by bacteria and fungi that leads to microbial growth and all these factors result in degradation of the genomic DNA in physical, chemical, and biological aspects [5]. Lately, many criminals dispose of their victims in watery graves in Malaysia and many cases require forensic DNA experts to process evidence from rivers, lakes, and sink pipes [6]. Trace evidence such as fabrics and blood play a vital role in investigation with DNA analysis as affirmed by van Oorschot et al. [7]. The aim of this research is to study the persistence of DNA extracted from different types of bloodstained fabric samples immersed in different types of water. This study is significant since the environmental factors in Malaysia are different from other countries which would affect the different types of water and eventually the DNA results. In addition, determination of how long the DNA will last in different types of water will be helpful to determine the estimated duration of the bloodstained dead body or only the bloodstained fabric that had been submerged in a specific type of Malaysian water.

EXPERIMENTAL

Collection of Water Samples for Immersion of Fabric Samples

Water samples used were lake water, collected from Tasik Seksyen 7, Shah Alam, Selangor (3.078464, 101.491004), seawater from Pantai Remis, Jeram, Selangor (3.201212, 101.305650), river water was obtained from Sungai Klang, Klang, Selangor (3.158633, 101.556577) and tap water which was collected from one of the residential homes in Seksyen 7, Shah Alam (3.079921, 101490759). 6 litres of water samples of each type were collected and stored in 6 litre labelled

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clean water bottles following the method recommended by Walden et al. [8] with slight modifications.

Determination of Conductivity, Dissolved Oxygen and Total Dissolved Solids

The four water samples sourced from the lake, river, sea, and tap were analysed for conductivity, dissolved oxygen and total dissolved solids parameters using a YSI multiparameter probe as recommended by Mohamed and Kheng [9]. The instrument was firstly calibrated for 5 minutes by immersing the sensor into the provided buffer solution. Approximately 1 litre of each water sample was poured into individual beakers. The sensor of the instrument was completely immersed in the lake water sample first. The readings were taken and recorded for conductivity (mS cm^{-1}, µS cm^{-1}), dissolved oxygen (mg L^{-1}, %) and total dissolved solids (g L^{-1}). The sensor was then cleaned and dried before testing the other water samples. The readings were recorded in triplicate for each water type.

Determination of Water Density

10 mL of lake water was placed in the pre-weighed measuring cylinder which was then weighed again to determine the mass of the water sample. The mass of water was obtained by subtracting the weight of the empty measuring cylinder from the weight of the measuring cylinder with 10 mL water sample. The same procedure was repeated for the other water samples and the water densities were recorded and tabulated. This procedure was performed in triplicate for each water type.

Sample Preparation

The ten types of pure fabrics namely satin, chiffon, linen, polyester, cotton, crepe, argenti, valentro, lycra and georgette were bought from the same store. The selected fabrics are commonly used in Malaysia and the fabrics were categorised into natural fabrics (cotton, satin, chiffon, linen) and synthetic fabrics (crepe, argenti, valentro, lycra, georgette, polyester). Each fabric sample were prepared in three replicates for each different types of water: lake water, river water, sea water, and tap water. Each fabric replicate was cut into square pieces about 10 cm by 10 cm. A pooled human blood source was obtained for this study and 40 ml was poured onto the 120 fabrics replicates to ensure that the fabrics were fully covered with blood. The fabric samples were then left drying at an average room temperature of 26 °C to 29 °C for 72 hours. After the drying process, 2 cm by 2 cm piece from each fabric replicates were cut using sterilized scissors to be used as standards. Then the fabrics were packaged into labelled paper envelope separately for each replicate and were preserved in -20 °C. These sample preparations were based on a previous study [10] with minor modifications.

Sample Collection

The total of 120 replicates of 8 cm x 8 cm dried bloodstained fabric samples were placed in separate plastic bags with 2 holes of 3 cm diameter following the method done in the study by Frippiat et al. [6]. Three new pails were used for each water type so all together there were twelve new pails used for four different types of water. The amount of water used for each pail in this experiment was limited to 6 litres. The pails were not closed to allow for daily weather conditions exposure as to mimic a crime scene. Sample collection was performed every 24 hours for 3 days. Each day, 2cm by 2cm pieces were cut from the bloodstained fabrics. The collected samples were air-dried and packaged into a separate labelled envelopes before being preserved at -20 °C as recommended by Hara et al. [11] to prevent DNA degradation during long-term storage. The surrounding temperature and water temperature were taken daily by using a digital and portable thermometer (Extech) while pH reading was taken daily using pH meter (Mettler Toledo) during samples collection. The readings were recorded in triplicate daily for each water type and the surroundings.

DNA Extraction

DNA extraction of the bloodstained samples was performed using a NEXprepTM NexK-3100 Genomic Mini Kit (Blood DNA Mini Kit, 100 prep). All the solution preparations and extraction steps were performed according to the manufacturer's instructions.

DNA Quantification

The DNA was measured using Eppendorf Bio-Photometer Plus. The measurement was taken without using the baseline solution as blank to mimic the real crime scene investigation. The wavelengths used were 230, 260 and 280 nm for concentration, purity, and contamination. 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 wavelength used for this measurement was 260 nm for nucleic acids detection, 280 nm for proteins and phenol and 230 for absorption of carbohydrates. The ratio of $A260/A280$ represents the purity of the DNA contained in the fabric sample from a range of 1.8 to 2.0, and if the value is less than 1.8, the DNA in the fabric sample is not pure. The ratio of $A260/A230$ represents the contamination of the DNA contained in the fabric sample and this value must be more than 2.0. A value of less than 2.0 indicates that the sample has been contaminated [12].

Table 1. Average temperature for each water and its surrounding.

Day	Surrounding temperature	Type of water			
		Lake water	River water	Sea water	Tap water
0	31.2 °C	30.1 °C	30.1 °C	30.1 °C	29.9 °C
1	30.5 °C	28.3 °C	28.3 °C	28.1 °C	27.8 °C
2	29.1 °C	28.2 °C	28.2 °C	28.0 °C	27.7 °C
3	31.0 °C	27.8 °C	28.0 °C	27.7 °C	27.5 °C

Table 2. Average water pH value for 4 different types of water.

Day	Type of water			
	Lake water	River water	Sea water	Tap water
0	7.00	7.16	7.21	7.42
1	7.32	7.65	7.45	7.43
2	7.58	7.82	7.63	7.64
3	7.97	8.00	7.74	7.84

RESULTS AND DISCUSSION

Parameters for Water Samples

The fabric samples containing the bloodstain were immersed in the four types of water for three consecutive days. Daily temperature and pH readings are taken from day 0 to day 3. According to Table 1, temperature of tap water was always the lowest compared to the other three types of water for each day meanwhile the other three types of water had roughly the same readings. It was also observed that surrounding temperature was always higher than the temperature of all the four water types. As shown in Table 2, the pH readings for all type of waters were neutral or slightly basic. Overall, the pH readings for all types of water were slowly increasing day by day to become more basic.

The electrical conductivity (σ), dissolved oxygen (DO) and total dissolved solids (TDS) of all the water samples were determined using YSI instrument. The electrical conductivity, σ is defined as the measure of the ability of a substance to transmit electric charge. According to Table 3, sea water had the highest conductivity followed by river water, then lake water and finally tap water. The measurement of DO concentrations indicates the presence of oxygen as the most readily available oxidizing agent and plays a major role in metabolic activities as well as ecological health of ecosystems. Tap water has the highest DO readings followed by lake water, then river water and finally sea water. Higher readings for total dissolved solids (TDS) show poor water quality. Sea water had the highest TDS reading followed by river water, then lake water and lastly, tap water.

All the four water samples have fairly the same densities with little differences. The decreasing order for the water density is lake water followed by river water then tap water and finally sea water, as shown in Table 4.

Table 3. Average readings of conductivity, dissolved oxygen, and total dissolved solids of water samples.

Water sources	Conductivity		Dissolved oxygen, DO		Total dissolved solids, TDS (g L^{-1})
	mS cm^{-1}	µS cm^{-1}	mgL^{-1}	%	
Lake	0.092	97	5.84	74.3	0.060
River	0.368	382	5.63	70.7	0.239
Sea	34.42	36344	4.33	62.3	22.38
Tap	0.090	95	7.16	91.1	0.059

Table 4. Average density of water samples

Water sources	Mass of water, (g)	Density of water, (g L^{-1})
Lake	9.8521	0.9852
River	9.8428	0.9843
Sea	9.4604	0.9460
Tap	9.8284	0.9828

Overall Comparison of DNA Concentrations in All Fabrics

DNA analysis is useful for solving a crime by identifying the source of biological evidence found at a crime scene [13]. Along with UV radiation and oxidation [14], water is an important factor affecting DNA concentration [6]. Processing DNA evidence from different types of bloodstained fabrics immersed in different types of water might provide valuable information on DNA persistence for DNA analysis. Seah et al. [15] highlighted that the presence of biological fluids, for instance, blood, semen, or saliva on fabrics such as clothing or bedding items is one of the most significant materials that serves as evidence recovered from a crime scene in Malaysia.

This research shows that all types of fabrics immersed in all types of water shows positive value for DNA concentration on day 1. The concentrations of DNA from all type of fabrics drastically decreased to almost half of the DNA concentration within 24 hours of immersion for all types of water. Table 5 shows that among the ten different types of fabrics immersed in lake water, three synthetic fabrics (georgette valentro, crepe) and three natural fabrics (cotton, satin, chiffon), retained positive value for DNA concentration until day 2.

Table 5. Average DNA concentration retained from fabrics immersed in lake water for 3 days.

Type of Fabric	Lake Water			
	Day 0	Day 1	Day 2	Day 3
	DNA concentration (mean ± S.D) (ng/µL)			
Cotton	340.7±2.71	195.6±2.35	37.4±0.31	-96.6±0.56
Satin	96.4±0.50	41.9±0.96	13.7±0.64	-43.3±0.44
Polyester	74.6±1.07	52.8±4.31	-35.7±2.07	-116.7±1.76
Chiffon	481.3±2.46	237.4±1.85	90.2±1.13	-362.3±1.37
Linen	134.8±5.40	69.0±1.72	-1.5±0.31	-37.3±0.60
Lycra	94.4±0.35	22.5±0.65	-24.0±1.13	-296.9±0.41
Crepe	204.5±1.27	147.2±1.31	10.6±0.12	-67.4±0.16
Argenti	159.8±1.97	114.8±2.04	-36.1±2.50	-81.2±0.82
Valentro	551.4±1.06	124.5±3.0	16.2±2.76	-26.8±2.96
Georgette	518.9±0.87	274.2±2.65	73.4±0.72	-16.3±0.81

Table 6. Average concentration of DNA retained from fabrics immersed in river water for 3 days.

Type of Fabric	River Water			
	Day 0	Day 1	Day 2	Day 3
	DNA concentration (mean ± S.D) (ng/µL)			
Cotton	210.8±0.36	179.0±1.95	-183.2±1.42	-218.9±0.50
Satin	220.4±2.17	101.3±4.51	81.1±0.10	-105.2±1.05
Polyester	46.6±0.70	14.6±1.31	-62.5±0.40	-271.8±0.17
Chiffon	653.7±5.96	383.6±4.68	-61.4±0.29	-103.5±0.55
Linen	329.9±1.72	183.5± 6.09	42.6±0.90	-51.2±0.69
Lycra	516.7±2.67	110.9±7.85	54.5±0.26	-89.8±0.75
Crepe	341.9±1.12	88.0±0.76	-67.1±0.85	-101.2±0.46
Argenti	478.8±2.19	220.3±1.33	-92.4±0.23	-175.6±1.37
Valentro	327.3±1.19	102.3±0.46	24.3±7.00	-11.9±0.81
Georgette	378.8±4.16	158.8±5.26	-8.9±0.26	-67.8±0.21

Table 7. Average concentration of DNA retained from fabrics immersed in sea water for 3 days.

Type of Fabric	Sea Water			
	Day 0	Day 1	Day 2	Day 3
	DNA concentration (mean ± S.D) (ng/μL)			
Cotton	523.5±0.58	134.4±1.91	31.7±0.41	-184.9±0.35
Satin	59.4±0.81	13.0±1.42	-188.1±1.62	-356.2±1.20
Polyester	213.7±4.19	70.2±0.29	-378.3±1.31	-433.6±0.58
Chiffon	165.1±1.85	8.5±0.31	-60.1±0.20	-180.0±0.66
Linen	224.9±8.89	12.7±0.67	-28.0±0.17	-325.1±3.45
Lycra	404.3±2.41	213.4±3.75	-8.6±0.20	-209.6±1.10
Crepe	405.8±1.77	52.3±0.16	-47.2±0.21	-107.7±0.52
Argenti	671.8±4.25	66.3±0.83	24.2±0.17	-29.4±0.51
Valentro	316.4±3.44	223.6±3.90	-16.2±0.45	-64.0±0.26
Georgette	395.3±3.32	103.7±1.32	63.1±0.10	-33.8±0.76

As shown in Table 6, the DNA concentration were decreasing drastically from day 1 to day 3 for fabric samples immersed in river water. Only satin and linen (natural fabrics) together with lycra and valentro (synthetic fabrics) retained positive value for DNA concentration on day 2.

DNA concentrations were significantly decreasing for fabric samples immersed in sea water as shown in Table 7. Only cotton, argenti and georgette fabrics retained positive value for DNA concentration on day 2.

Most fabrics immersed in tap water showed positive value for DNA concentration up to day 2. As observed in Table 8, only fabric cotton, chiffon, linen, lycra and valentro shows negative value for DNA concentration on day 2. This research also found that synthetic fabrics like crepe, argenti, valentro and georgette have better retention for DNA concentrations compared to natural fabrics as discovered by Kulstein and Wiegand [16].

On the other hand, the finding shows that all types of fabrics immersed in all types of water shows negative value for DNA concentration on day 3. The most probable explanation of the negative readings for DNA concentration was due to sample contamination and the measurement was taken without using the baseline solution as blank to mimic the real crime scene investigation.

Based on this research fabric samples immersed in tap water have better retention for DNA concentration followed by lake water, river water and lastly sea water. This finding corresponds with the study done by Graham [17] that DNA loss is higher in salt water than in fresh water. The most reasonable explanation of DNA loss in river water and lake water is because both this water is a habitat for living organisms which lead to presence of animal and plant wastes. The presence of organic compounds due to decomposition the animals and plants and various synthetic organic compounds such as detergents, solvents and pesticides which originated from agricultural residues and wastes disposed from domestic and industrial areas can also cause DNA degradation. However, tap water is filtered and undergoes several processes to produce water that is suitable for domestic use and safe for consumption particularly by human beings [4].

Table 8. Average concentration of DNA retained from fabrics immersed in tap water for 3 days.

Type of Fabric	Tap Water			
	Day 0	Day 1	Day 2	Day 3
	DNA concentration (mean ± S.D) (ng/μL)			
Cotton	140.4±0.78	44.3±0.91	-307.3±0.45	-401.2±1.00
Satin	468.6±2.41	287.8±5.98	71.1±0.23	-10.1±0.29
Polyester	485.3±0.91	144.7±3.56	93.5±3.26	-86.4±1.35
Chiffon	478.1±0.65	158.8±0.50	-34.7±1.33	-383.4±1.46
Linen	235.7±1.67	53.7±2.46	-10.6±0.10	-320.5±0.26
Lycra	77.6±0.36	44.2±0.51	-29.0±0.45	-61.5±0.51
Crepe	403.3±2.55	182.4±3.09	49.5±0.35	-2.5±0.38
Argenti	82.2±1.34	20.7±0.21	8.5±0.91	-24.9±0.51
Valentro	656.2±2.92	337.5±8.33	-52.2±0.64	-98.1±0.86
Georgette	478.5±0.38	161.8±0.81	72.3± 2.82	-43.1±0.98

Figure 1. Condition of the fabric before immersion in tap water.

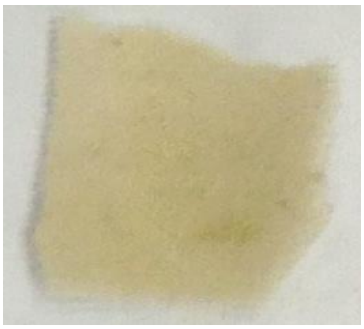

Figure 2. Condition of the fabric after 24 hours of immersion in tap water.

Even though, Graham [17] highlighted that 72 hours' time is very significant in the timeline of DNA loss of human tissue and bone immersed in aqueous environment and Frippiat et al. [6], showed rapid blood degradation occurs is 72 hours and highlighted that underwater DNA degradation is likely dependent by many factors but this research shows that the average concentrations of DNA from all type of fabrics were drastically decreased over the period of three days experiment with almost half of the DNA loss occurred within the first 24 hours of immersion in water, as shown in Figure 1 and Figure 2.

Purity of DNA and DNA Contamination

This research also found that the extracted DNA for all types of fabrics after the first 24 hours of immersion in all types of water gives value less than 1.8 indicating the DNA sample is not pure. This finding further supported with the value less than 2.0 for DNA contamination that shows the extracted DNA after the first 24 hours from all types of fabrics immersed in all types of water have been contaminated. The low ratios of A260/A230 may be caused by a contaminant absorbing at 230 nm or less and low ratios of A260/ A280 may be the result of very low nucleic acid concentration [18].

CONCLUSION

In conclusion, it was found out that the fabrics that were immersed in tap water have the highest DNA concentrations indicating that these fabrics also have the lowest DNA degradation followed by lake water, river water and sea water. It was also discovered that among the ten types of fabrics, synthetic fabrics have better retention for DNA concentrations compared to natural fabrics. The concentrations of DNA from all type of fabrics were drastically decreased over the period of three days with almost half of the DNA concentration reduced within 24 hours of immersion for all types of water. This study also showed that underwater DNA loss and DNA degradation is very high within the first 48 hours compared to 72 hours reported in previous studies. Future studies are recommended to shorten the time of fabrics collection to hourly instead of daily so that it could provide more valuable information for crime investigations.

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REFERENCES

1. Gupta, M., Saran, V., Mishra, M. K. and Gupta, A. K. (2016) Examination of traces of blood stains

on different fabrics after washing. *International Journal of All Research Education and Scientific Methods*, **4(6)**, 204–209.

2. Mohite, P. M., Keche, A., Anjankar, A. J. and Ninave, S. (2011) Effect of ageing & environmental condition for detection of blood group from blood stain. *Journal of Indian Academy of Forensic Medicine*, **33(4)**, 308–310.

3. Alice, P., Audrey, E., Martine, B., Guillaume, M., Antoine, D. and Christian, D. (2016) Persistence of stains and DNA evidence in hostile situations. *Forensic Science Today*, **2(1)**, 10–12.

4. de Oliveira Musse, J., da Costa Nardis, A., Anzai, E. K., Hirata, M. H., Cicarelli, R. M. B. and de Oliveira R. N. (2009) Freshwater and salt-water influence in human identification by analysis of DNA: an epidemiologic and laboratory study. *Brazilian Journal of Oral Sciences*, **8(2)**, 71–75.

5. Bender, K. and Schneider, J. P. (2004) Preparation of degraded human DNA under controlled conditions. *Forensic Science International*, **139(2-3)**, 135–140.

6. Frippiat, C., Gastaldi, A. and Grunderbeeck, S. V. (2017) Persistence of immersed blood and hair DNA: A preliminary study based on casework. *Journal of Forensic and Legal Medicine*, **51**, 1–8.

7. Van Oorschot, R. A. H., Ballantyne, K. N. and Mitchell, R. J. (2010) Forensic trace DNA: a review. *Investigative Genetics*, **1**, 14.

8. Walden, C., Carbonero, F. and Zhang, W. (2017) Assessing impacts of DNA extraction methods on next generation sequencing of water and wastewater samples. *Journal of Microbiological Methods*, **141**, 10–16.

9. Mohamed, K. and Kheng, L. K. (2012) Methods of Analysis for Water and Wastewater. *Percetakan Haji Jantan Sdn. Bhd., Cheras Kuala Lumpur*, 6–7.

10. Thabet, H. Z., Ghandour, N. M. and Salama, R. H. (2018) Effect of some cleaning products on blood DNA retrieval from cloth. *Egyptian Journal of Forensic Sciences and Applied Toxicology*, **18(2)**, 53–62.

11. Hara, M., Nakanishi, H., Tamura, A., Yoneyama, K., Saito, K and Takada, A. (2015) Effects of storage method on DNA degradation in old bloodstain samples. *Forensic Science International: Genetics*, **5**, 39–41.

12. Hanno, K. K., Ihle, M. A. and Kubasch, R. (2015) Troubleshooting Guide for the measurement of Nucleic Acids with Eppendort BioPhotomoter® D30 and Eppendorf Bio-spectrometer®. Eppendorf AG, Hamberg, Germany.

13. Dissing, J. (2010) Exploring the limits for the survival of DNA in blood stains. *Journal of Forensic and Legal Medicine*, **17(7)**, 392–396.

14. Hall, A., Sims, L. M. and Ballantyne, J. (2014) Assessment of DNA damage induced by terrestrial UV irradiation of dried bloodstains: forensic implications. *Forensic Science International: Genetics*, **8(1)**, 24–32.

15. Seah, L. H., Othman, M. I., Jaya, P. and Jeevan, N. H. (2004) DNA profiling on fabrics: An in-situ method. *International Congress Series*, **1261(C)**, 565–567.

16. Kulstein, G. and Wiegand, P. (2017) DNA/RNA co-analysis of seminal fluid-stained fabrics after water immersion for up to seven days. *Forensic Science International: Genetics*, **6**(September), e27–e28.

17. Graham, E. (2013) Effects of different types of water on the degradation rate of human DNA in bone and tissue. *Ph.D. Diss. University of New Haven, Connecticut*.

18. Matlock, B. (2015) Assessment of nucleic acid purity. *Thermo Fischer Scientific*, 1–3.