The Stability of Semen Stain on Different Kinds of Fabric Buried on Soil

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Abstract The forensic examination of semen is essential for criminal investigations in sexual assault cases. However, detecting semen on clothing is often challenging, especially when the body has been buried, due to environmental degradation and fabric type. Our study evaluates semen stain stability on different fabrics buried in soil. Semen samples were applied to cotton, polyester, and denim fabrics and buried in soil. Samples were collected at 0, 24, 72 hours, 7 days, and 14 days post-burial. Acid phosphatase (AP) activity and sperm count were analyzed as semen markers. Results indicated that both markers were detectable across all fabric types but exhibited varying degradation rates. Polyester showed the fastest decline in semen markers compared to cotton and denim fabrics. Environmental conditions, such as humidity, soil moisture, and pH, significantly influenced the persistence of semen evidence. Elevated humidity levels were associated with reduced AP activity, indicating more rapid degradation. These findings highlight that fabric type and environmental conditions critically impact semen stain retention and marker stability. The timing of evidence collection is crucial for reliable detection. Forensic investigations in sexual assault cases involving buried evidence should incorporate these variables to enhance the interpretation of semen component persistence and support justice outcomes.

Keywords: Forensic science, Acid phosphatase, Sperm, Semen

1. Introduction

In sexual crime investigations, semen stains and semen-contaminated objects constitute vital elements of important forensic evidence [1]. Semen stains can be found on the body of the victim's body in location, such as in the vaginal area, on the chest, in the mouth, or around the anus. Likewise, semen stains are also found on the clothes of the victim as well. These are tiny points that are not visible to the naked eye. Typically, sperm can survive on the body for up to 48–72 hours and may persist up to two weeks without cleaning [2]. In cases of sexual assault where the victim has passed away, sperm can remain in the vaginal cavity for 3–5 days, and sperm traces on clothing or other such as tiles surfaces can last for several months without washing [3, 4]. In sexual assault evidence analysis, the Acid Phosphatase (AP) test is commonly employed due to its specificity for an enzyme found abundantly in the prostate gland [5]. Another method used is the detection of prostate-specific antigen (PSA), a protein component of semen, which can also be identified in vasectomized individuals. This immunochromatographic test relies on a sandwich complex of antibody-antigen-antibody formation in the presence of PSA in the sample [6]. Additional confirmation of semen presence can be conducted through staining methods such as Hematoxylin and eosin, Kernechtrot-Picro-indigo-car-mine (KPIC), or Oppitz staining to observe sperm [7].

However, several factors impact the stability of semen stains, including time, temperature, humidity, sunlight, environmental conditions, the material of the substrate, and the cleaning process. Clothing worn by the victim may contain semen stains, which vary in retention depending on the fabric type, with some cases showing a loss of detectable traces over time. Moreover, perpetrators may attempt to conceal evidence by discarding, burning, or burying the victim's clothing to hinder investigation [1]. In previous studies examining the persistence of semen on five types of fabric underwater exposure (tap water, river water, pool water, and canal water) over 14 days, it was found that the stability of semen stain varied by fabric type. Linen retained detectable semen traces for only up to 72 hours, showing lower stability compared to other fabrics.

In ongoing rape cases, the occurrence is often unpredictable, with perpetrators not discriminating by the victim's clothing material, and victims are unable to select their attire at the moment. In natural environments, particularly soil, the mineral composition may affect the stability of semen stain components [8]. Previous research has a limited

document to show the stability of semen components on fabrics buried in soil. Knowing the duration and stability of semen components in different environments could inform laboratory diagnostics. For instance, an absence of components such as AP and PSA in the victim's clothes which were buried in soil does not rule out the occurrence of sexual assault; environmental factors like soil composition, fabric type, and burial duration could degrade these components. Therefore, forensic laboratories may need alternative testing methods under these circumstances.

This research aims to study and compare the stability of semen stains on various types of fabrics buried in soil over two weeks, aligning with the knowledge that semen can persist for up to two weeks in the human body. The study will examine intervals at 24 hours, 72 hours, 7 days, and 14 days to assess how long semen stains remain detectable on different fabrics in soil, aiding in victim support and corroborating evidence against perpetrators for legal proceedings toward justice.

2. Materials and Methods

2.1 Sample collection

This study was ethical approval from the Human Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (No. 453/2566, study code NONE-2566-0426) for the collection of human semen samples. A single healthy Thai male volunteer provided the semen sample by masturbation. The semen sample was collected in a sterile sealed plastic container and immediately delivered to the laboratory for analysis. The acceptance criteria for semen samples included a minimum volume of 1.5 milliliters per collection, a pH level of 7.2 or higher to indicate slightly alkaline conditions, and a white, cloudy appearance. Deviations in color, such as yellow-green or pinkish brown, would suggest possible infections or blockages within the reproductive tract.

2.2 Seman stain on fabric

Three types of fabrics consisted of cotton, polyester, and denim fabric were chosen for our study. Fifty microliters of semen were dropped into a fabric with the size 2 x 2 cm^2. A total 45 of fabric was divided into 3 groups according to the time for collection (3 pieces of fabric for each collection time). After the semen was dried on the fabric, it was placed in a plastic box containing soil. All fabrics were buried 25 cm. in death from the top. The box was placed in an open environment. Each semen-stained fabric was harvested at 0 hours, 24 hours, 72 hours, 7 days, and 14 days for semen analysis.

2.3 Semen extraction

The sperm and semen's components from the fabric buried in the soil was extracted by soaking the fabric in a test tube containing 1 ml of 0.9% sodium chloride (NaCl) solution to dissolve the semen stain and preserve the sperm and its components. After incubation for 30 minutes, the fabric was removed and the extracted solution was transferred into a microcentrifuge tube [7]. After centrifugation at 6,000 rpm for 5 minutes, the supernatant was collected for testing the enzyme acid phosphatase (AP) and the sediment for counting the sperm present in the sample under a microscope. To prevent the contamination during the extraction process, all lab equipment is disinfected with sterilization process by autoclave or disinfectant with alcohol.

2.4 Quantification of acid phosphatase (AP) activity

To measure the level of AP activity from semen, buffer substrate containing sodium thymolphthalein monophosphate and citrate buffer [9] was incubated at 37˚C for 5 minutes. Then, a semen sample obtained from the extraction was added into the tube. After incubation at 37˚C for 30 minutes, the color developer consisting of sodium hydroxide and sodium carbonate anhydrous was added into the tube. Finally, the absorbance of color reaction was measured at a wavelength of 590 nm [10] The activity of AP was calculated by comparing with the standard graph and represented as unit per liters (U/L). To avoid the interference of the enzyme, all reagents were freshly prepared before performing an experiment and stored in amber bottle.

2.5 Sperm counting

To measure the amount of sperm in the semen stain, the sediment form extraction process was dissolved with 0.9% sodium chloride (NaCl) solution. Then, the solution was filled into hemocytometers. The sperm was counted under a light microscope. The amount of sperm was calculated as previously described [11] and represented as cell per milliliter (cells/mL).

2.6 Statistical analysis

Statistical analysis was conducted using IBM SPSS for Windows, version 20. The experimental results are presented as means and standard deviations. One-way ANOVA with Bonferroni analysis was used to compare the differences between groups of normally distributed data. For non-parametric data, Kruskal-Wallis analysis was used to compare the sperm counts of the different categories. The relationship between AP activity and sperm counts as well as environmental condition was investigated by Pearson's (parametric data) and Sperman's (non-parametric data) correlation. Additionally, a linear regression analysis was conducted to investigate the influenced of environmental condition AP activity and sperm count, with a 95 percent confidence level ($p<0.05$).

3. Results

The weather conditions including temperature, rainfall, sunlight, relative humidity and soil moisture throughout the study were referenced from the Northern Meteorological Center, Mueang Chiang Mai District, Chiang Mai

Province. The soil samples were also sent to the laboratory at the Faculty of Agriculture, Chiang Mai University for measuring pH. The result is presented in Table 1

Table 1 The environmental condition during the study.

Time	Temperature (°C)	Rainfall (mm)	Sunlight (Hrs.)	Relative humidity (%)	Moisture content soil (%)	pH
0 hour	37.3	0	10.2	45	7.56	6.47
24 hours	37.3	0	10.2	45	9.47	6.45
72 hours	32.6	0	8.8	51	8.14	6.41
7 days	29.8	1.4	5.4	57	7.24	6.47
14 days	32.6	0	9.4	47	3.33	6.52

Initially, the AP activity and sperm count in semen stains on cotton, polyester, and denim fabrics showed no significant differences. When comparing enzyme activity at different time points, all three fabric types exhibited a significant decrease in AP activity after 24 hours, 72 hours, 7 days, and 14 days compared to 0 hours. Moreover, the enzyme activity in semen stains on polyester appeared to be the lowest compared to stains on cotton and denim fabrics at 24 hours, 72 hours, 7 days, and 14 days. The sperm count in semen stains on cotton significantly decreased after 24 hours. However, no significant decrease in sperm count was observed for semen stains on polyester and denim fabrics at this time. A significant decrease in sperm count for semen stains on polyester and denim fabrics was observed at 7 and 14 days. From these results, it is assumed that denim fabric initially preserves higher AP activity and sperm count but does not sustain them as well as cotton over time. Polyester shows the poorest retention of both AP activity and sperm count, suggesting its material properties may contribute to faster degradation. The result is shown in Table 2.

Table 2 The activity of AP activity and sperm count derived from semen stains on cotton, polyester and denim fabric buried in soil are reported as mean values ± standard deviation.

Time	Cotton		Polyester		Denim	
	Acid phosphatase (U/L)	Sperm count (cells/mL)	Acid phosphatase (U/L)	Sperm count (cells/mL)	Acid phosphatase (U/L)	Sperm count (cells/mL)
0 hrs.	139.28 ± 15.64 (a)	$4.41\times10^{6} \pm 2.63\times10^{5}$ (b)	137.03 ± 11.66 (a)	$4.80\times10^{6} \pm 4.22\times10^{5}$, (b)	144.20 ± 4.56 (a)	$5.61\times10^{7} \pm 2.00\times10^{6}$ (a)
24 hrs.	27.20 ± 5.60*, (a)	$2.52\times10^{6} \pm 2.52\times10^{5}$* , (b)	2.99 ± 0.51*, (b)	$1.19\times10^{6} \pm 4.62\times10^{5}$, (a)	54.26 ± 7.61*, (c)	$4.50\times10^{7} \pm 4.34\times10^{6}$ (b)
72 hrs.	1.60 ± 0.23*, (a)	$4.54\times10^{5} \pm 1.37\times10^{5}$, (a)	0.29 ± 0.09*, (b)	$2.31\times10^{5} \pm 1.62\times10^{4}$, (b)	0.65 ± 0.12*, (c)	$2.59\times10^{5} \pm 4.28\times10^{4}$ (b)
7 days	0.40 ± 0.10*, (a)	$1.20\times10^{5} \pm 4.26\times10^{4}$*, (b)	0.28 ± 0.03*, (b)	$7.41\times10^{4} \pm 1.60\times10^{4}$*, (a)	0.30 ± 0.03*, (b)	$1.11\times10^{5} \pm 2.79\times10^{4}$*, (b)
14 days	0.17 ± 0.04*, (b)	$6.48\times10^{4} \pm 1.60\times10^{4}$*, (b)	0.19 ± 0.03*, (b)	$3.71\times10^{4} \pm 1.61\times10^{4}$*, (a)	0.27 ± 0.04*, (a)	$7.41\times10^{4} \pm 1.60\times10^{4}$*, (b)

Asterisk (*) represented the statistical significance between each time point relative to the baseline (0 hours). The alphabet (a, b and c) represents the statistical significance between type of fabric at the same time point at a p-value < 0.05.

To clearly show the degradation rate of AP activity and sperm count, the remining percentage of AP activity and sperm count at each time for collection was calculated relative to the enzyme activity or sperm count at 0 hours. The finding illustrates a time-dependent decline in the percentage of remaining AP activity over 14 days. We found that the activity of enzymes in polyester seems to sharply decrease within the first 24 hours. During 24 – 72 hours, the high degradation rate of enzyme activity was found to be the semen stain on denim (Figure 1). Across all conditions, AP activity declines over time, however the rate and extent of degradation differ. AP on polyester represents the fastest and most complete degradation, while AP on denim shows the slowest degradation rate. For sperm count, the number of sperm in polyester seems to sharply decrease within the first 24 hours. During 24 – 72 hours, the trend of sperm degradation rate was similar between cotton and denim fabrics. Also, the high degradation rate of sperm was remarkably observed in the denim fabrics after 72 hours (Figure 2). It suggests that sperm count on polyester shows the fastest and most complete degradation, while sperm on denim fabric reflects a more gradual reduction.

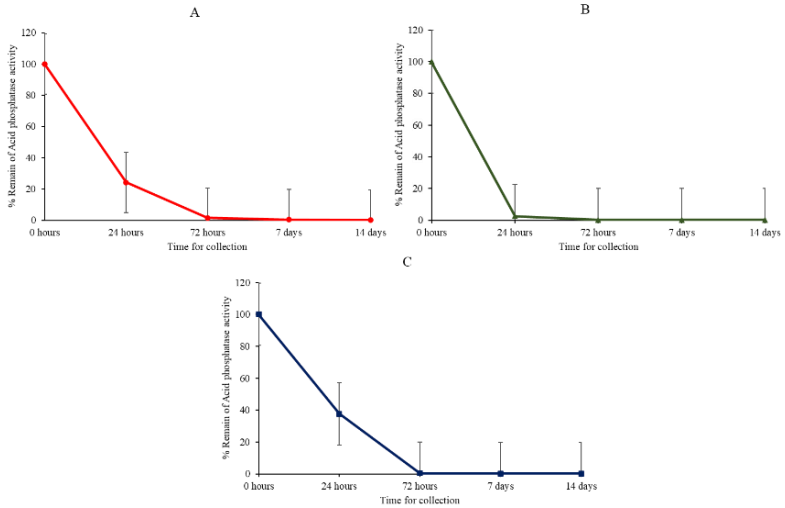
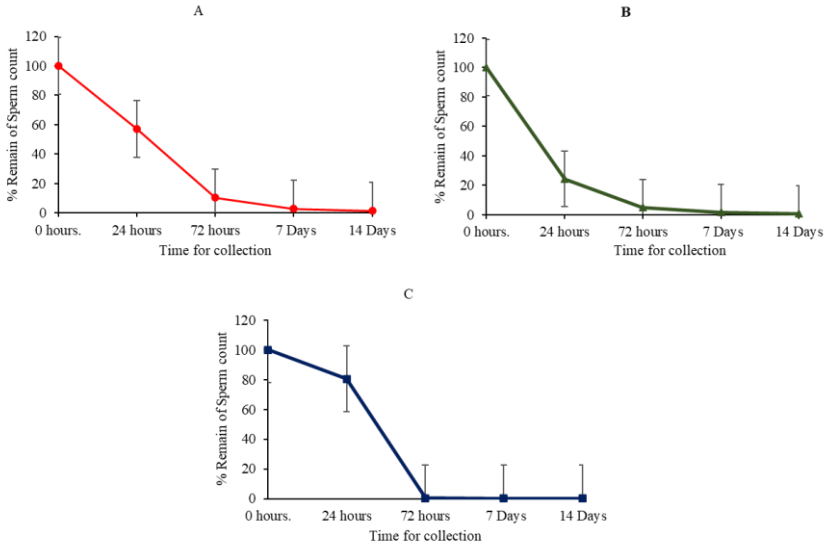


Figure 1 The remaining percentage of AP activity at each time for collection was calculated relative to time 0 hours. (A) cotton fabric, (B) polyester fabric, and (C) denim fabrics.



Figures 2 The remaining percentage of sperm count at each time for collection was calculated relative to time 0 hours. (A) cotton fabric, (B) polyester fabric, and (C) denim fabrics.

To demonstrate the correlation between the environmental condition and test substances (AP activity and sperm count) on fabrics, the Pearson correlation coefficient and Spearman's rho correlation coefficient was calculated. We found an inverse relationship between the enzyme activity and time for collection of all three types of fabrics (r = -0.458 for cotton, r = -0.458 for polyester and r = -0.461 for denim fabrics). Likewise, the significance of inverse relationship was found between the enzyme activity and humidity for all three types of fabrics with the correlation coefficient by -0.485, -0.465, and -0.495 for cotton, polyester, and denim fabrics, respectively. Besides, the significant

relationship between the enzyme activity in semen stain on denim fabrics was direct proportional to sunlight (r = 0.389). In addition, a positive relationship between the sperm counts and soil moisture was found to be semen stain on cotton (r = 0.685), polyester (r = 0.686), and denim fabrics (r = 0.684), whereas the sperm count was significantly negative correlation with soil pH (r = -0.450 for cotton, r = -0.450 for polyester, and r = -0.448 for denim fabrics).

After we found the correlation between the environmental condition and test substances (AP activity and sperm count) on fabrics, the further examination investigated the influence of environmental conditions on AP activity and sperm count by multiple linear regression analysis. AP activity and sperm count on three fabric types have been considered as dependent variables, while daily environmental conditions (including time, temperature, precipitation, sunlight, relative humidity, soil moisture, and soil pH) were classified as independent variables. Both time and relative humidity had a significantly negative association on AP activity on cotton, polyester, whereas one factor (time) was significantly negative association with the enzyme activity. It means that the AP activity decreased when time for collection and relative humidity were increased. Environmental conditions, specifically soil moisture and soil pH, were significantly positively associated with influenced sperm count in cotton and denim fabrics. It assumes that a reduction in soil moisture and pH is likely to result in a decrease in sperm count. In the case of polyester fabric, only soil moisture was the sole factor that significantly affected sperm count (Table 3).

Table 3 The association between AP activity and sperm count as well as environmental condition throughout the study. The result is shown as B ± Standard error

Fabrics	Environment	Acid phosphatase activity (U/L)	Sperm count (Cell/ml)
Cotton	Time for collection	**-0.905 ± 0.312**	-
	Relative humidity	**-6.730 ± 2.179**	-
	Soil moisture	-	**$9.07x10^5 \pm 2.33x10^5$**
	pH	-	**$3.80x10^7 \pm 1.35x10^7$**
Polyester	Time for collection	**-0.915 ± 0.320**	-
	Relative humidity	**-6.492 ± 2.231**	-
	Soil moisture	-	**$6.87x10^5 \pm 2.90x10^5$**
	pH	-	$3.26x10^6 \pm 1.68x10^7$
Denim	Time for collection	**-1.133 ± 0.371**	-
	Sunlight	11.709 ± 11.587	-
	Relative humidity	-2.396 ± 5.205	-
	Soil moisture	-	**$1.58x10^7 \pm 2.89x10^6$**
	pH	-	**$6.31x10^7 \pm 1.67x10^8$**

Correlation values that are significantly different ($p < 0.05$) are shown in bold.

4. Discussion

Semen stains and semen-contaminated objects are crucial forensic evidence in sexual crime investigations, often found on the victim's body or clothing, though many are invisible to the naked eye. Sperm survive on the body within the short period or may persist for months on fabrics. The environmental factor as well as the stability of semen's composition might interfere with the analysis process. This study examines the stability of semen on three commonly used fabrics—cotton, polyester, and denim—selected for their prevalence, durability, and suitability for various conditions. Three fabric types including cotton, polyester, and denim fabrics that are often worn by both men and women were chosen for this research because they are breathable, aesthetically pleasing, wrinkle-resistant, and appropriate for all seasons.

In this study, long-term persistence of AP activity on cotton, polyester, and denim fabrics was investigated. It was found that AP activity of in polyester fabric has significantly decreased after 24 hours of burial, whereas the enzyme activity was decreased after 72 hours of burial. Previous study stated that cotton and denim did not differ significantly in their ability to preserve sperm, while polyester was significantly different from both types of fabric. This could be because polyester is a synthetic material made from humans [1] with ethylene glycol and terephthalic acid via petrochemical processes is not a natural fiber from these plastics characterized by toughness and small pores ranging from 10 to 30 nanometers and thus retains only a small amount of AP activity [12], compared to cotton and denim fabrics, resulting in minimal retention of semen and sperm residue [12-14]. Polyester has substantially poorer moisture management characteristics, in terms of transporting moisture vapor and liquid away from the body when compared to cotton. In addition, polyester exhibits a moisture recovery less than cotton. Hence, The inadequate of

absorption of water and moisture of polyester [15] also reflect the capacity to hold semen.[13, 14]. In addition, the structure and composition of cotton fabric which is made of multiple layers of cellulose and intricately woven in a helical structure, possess pores approximately 50 to 100 nanometers in size, enabling effective is very proficient in retaining and absorbing seminal fluid [16], absorption and retention of semen [16, 17]. Denim fabric, a type of cotton-based fabric, is less soft than standard cotton due to its tight weaving structure, involving at least two parallel threads. The tighter weave reduces pore size to a level smaller or comparable to cotton. Additionally, if denim incorporates synthetic fibers such as polyester or elastane, the pore size decreases further, ranging between 20 to 80 nanometers [18], potentially allowing for absorption levels similar to cotton and affecting the detection efficiency of acid phosphatase enzyme activity. Previous research comparing the properties of different fabric types found that knitted fabrics and cotton twill have a high tendency to absorb water, whereas fur fabric and polyester exhibit low water absorption tendencies. Regarding liquid evaporation efficiency, the tested fabrics showed no significant differences, except for fur fabric, which demonstrated the lowest evaporation efficiency[19]. Moreover, cotton fabric shows excellent retention of semen stains, even after washing. For instance, a study by Nolan et al. (2018) detected sperm cells on cotton and terry cloth fabrics even after undergoing six washing cycles [20]. Previous research showed the enzyme activity on semen which was dropped into five different fabric types containing khaddar, silk, chiffon, polyester, and linen could still be counted for up to seven days after being immersed in water for 14 days. The khaddar, a handwoven natural fiber made from cotton, silk, and wool, retains enzyme activity for up to 14 days compared to polyester which displayed measurable enzyme activity for four days [1]. According to the previous finding, it supports our result that cotton fabrics woven from natural fibers, is more effective in retain AP activity more efficiently than polyester fabric.

According to figures 2, the percentage of remaining sperm in polyester fabric significantly decreased at 24 hours of burial, whereas the cotton and denim fabrics were observed after 72 hours of burial. It shows a similar reduction in sperm count in cotton fabrics seminal stains are retained more effectively than in denim. The research by Panicker et al., 2022 [21] found that cotton is better than denim in detecting and analyzing the amount of sperm. Cotton's porous nature enhances sperm extraction and increases sperm density visibility. On the other hand, sperm visibility is decreased by the closely woven and colored structure of denim. Denim's color has the potential to hide evidence over time, making investigations on this fabric less reliable. This research revealed that, on microscopic evaluation, polyester fabric resulted in the most significant reduction in sperm count within 24 hours among the three fabric types examined.

The analysis of environmental factors on enzyme activity and sperm count found an inverse correlation between time and humidity with enzyme activity, indicating that increases in time and relative humidity resulted in decreased AP activity in cotton, polyester, and denim fabric. Previous publication shows that the activity of AP in the semen stain will gradually decrease due to enzyme degradation [21]. Moreover, our finding demonstrated that sunshine exposure was identified as an additional variable influencing AP activity, especially on denim fabric. According to research in environmental substance such as humic compounds may have an impact on forensic tests that utilize enzymes. Humic materials in soil may bind to phosphatases and inhibit their action, which might result in less or delayed enzyme detection [22]. In the sperm count, soil moisture and pH showed a positive relationship. The reduction in soil moisture and soil pH may profoundly affect sperm quality and functionality. Previous studies suggest that low pH conditions negatively impact sperm motility and fertilization. The research conducted by [23] shows that soil moisture significantly influences the presence of endocrine-disrupting chemicals, which may imitate hormones and negatively impact male reproductive health. Reduced soil moisture and pH levels may increase the concentration of these disruptive chemicals, contributing to a deterioration in sperm quality and function. The combined impact of diminished pH and moisture levels may further impair sperm viability and overall fertility rates, as shown by relates to between environmental variables and reproductive health outcomes [24].The research conducted by Andrea and Carlo (2019) demonstrated that soil moisture and microorganisms contribute to sperm degradation, with prolonged exposure reducing sperm viability and damaging its functionality[23-25]

5. Conclusions

The study concluded that the preservation of semen stains varied depending on the type of fabric. In addition to the fabric type, environmental conditions such as humidity, moisture, soil pH, and the time of collection also influenced the detection of semen components, particularly sperm and acid phosphatase (AP) activity. Therefore, forensic investigations and the interpretation of semen analysis in sexual assault cases should consider these factors, especially when evidence is buried in soil.

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