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A forensic study on lifespan of blood stains on different soil in different temperature

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Abstract

Present research is an attempt to study the lifespan of the bloodstains on different soil in different temperature. India is a subcontinent, which has all type of vegetation and climate hence, it is necessary to know the lifespan of bloodstain spilled during a violent crime on land. The land can be any type viz, wetland, dryland, sand dune etc. Current study has been conducted by simulating the three different temperatures on 5 different types of soils collected from varied part of the state of Karnataka in India. The findings of the study lead us to the understanding of lifespan especially with blood grouping, blood stain exposed to three different temperatures viz, room, cold and heat and observation period was every 5th day. It has been found that the best evidence retraction temperature is cold irrespective of soil type and when it comes to the best soil type for retraction of blood stain was sand and agriculture soil which gave the best result than any other soil respectively.

Keywords: Blood Stain, Absorption Elution, Blood Grouping, Soil, Temperature.

Introduction

Forensic science investigates almost types of evidences in which biological specimen is a significant one when crime is against human body, in such scenario one of the important biological specimens might be blood. Hence, blood can be considered one of the key pieces of evidence found at scene of crime, deceased or victim and it will be considered significant and documented as well as sent for analysis by collecting the blood from scene of crime. Blood plays a major role in identifying the individual through the means of blood grouping to DNA analysis. The result will be positive only when it is free from contamination and any such influence of foreign bodies. Blood samples were utilised in identification of individuals in crime cases, accident cases, paternity disputes etc[1-4].

The crime scene and conditions at which biological stains are found can vary with each crime. Broadly the crime scenes which are found can be broadly classified into indoor crime scenes and outdoor crime scenes. Biological evidences can be contaminated and sometime damaged or loses its evidentiary value due to the exposure in outdoor crime scene when left unattended over a period of time. Environmental factors like rain, snow, heat, wind etc, will impact the biological evidences if it is not protected.

Hence one such scene where blood can be found will be on soil. Thus, the outdoor crime occurrence place can be a good source of evidence particularly in homicide cases when blood spilled over ground and the collection of soil form such crime scene might yield and connect the case with the culprit for identification. As soil differs to each place and its elements the organic comparison of such soil with blood stain might affect the quality and quantity of the evidence[5-10].

Biological evidences including bloodstains at crime scene can easily be contaminated if due care is not taken. Biological evidences might degrade due to the reasons like heat, moisture, microorganisms, etc. Moreover, deterioration of biological evidence can also be traced when it comes in contact with any chemicals. Forensic examination related to the bloodstains demonstrate that the pH factors can also be identified[11].

Numerous studies provide insight that there is a possibility of extracting results from the aged biological traces over a time. However, very few study hastried to quantify and qualify to study the blood stain lifespan on soil is very less hence, this is an attempt to critically analyse the life span of blood present on different types of soils at different temperatures. Thus, anexperiment was been undertaken to accurately determine whether the blood stain can be grouped even after exposure to different temperature in different soil[12].

The primary object of the study is to see to what extent adverse conditions such as temperature and time span would affect the blood group and to find out the extent of degradation of blood[13,14].

Materials and methods

Collection of samples: For the present study, two types of samples have been chosen i.e., blood and soil.

Soil: Soil for the present study has been chosen from 5 different places from the state of Karnataka viz, Sand from sea shore of Mangalore, Forest soil / Wet Land soil from Western ghats of Hassan, Agriculture Land soil from Mandya, Soil sample from Mechanic shop and from Construction site of Mysore & Bangalore respectively.

Blood: The major requirement for this research study was blood and samples were collected from blood bank in Mysore. On the basis of need for study A, B and Ogroup bloods were collected. Approximately 40ml of each blood samples were collected and preserved with EDTA in an airtight tube. They were then placed in CPDA (Citrate Phosphate Dextrose Adenine solution) bags and stored at 4^0 Celsius in refrigerator. Immediately analysis is done after blood samples are collected and remaining samples were preserved in refrigerator.

Soil Analysis: The five soil samples collected were sent to the Soil Health Centre for micro nutrient analysis. The soil samples were analysed for pH, EC, and major nutrients viz, Nitrogen, phosphorus and potassium, and organic Carbon.

Temperatures set for study: The room temperature was fixed to 22^0 Celsius has the minimum temperature was set to 5^0 Celsius for cold and maximum was 45^0 Celsius for hot temperature respectively.

Absorption Elution Techniques for the Identification of Blood Groups: ***Preparation of Blood Cells:*** A, B and O blood were collected prior to cells preparation and were stored in EDTA tubes at 4^0 Celsius. From 500 micro litres to 1 ml and were taken into 3 centrifuge tubes and were marked accordingly. 10ml of saline was then added to this and mixed well. This was then centrifuged for 8 min at 2500 rpm. After centrifuging the supernatant was discarded and the pellet was again mixed with 10 ml saline and mixed and centrifuged once again (6min;2500 rpm). This was again repeated once more. The supernatant was discarded, and the final solution was mixed with saline and a drop of this was used for analysis.

Blood sample analysis: i. Nine clean and dry test tubes were taken for each soil sample and marked as Room (A, B, and O); Cold (A, B, and O); Heat (A, B and O). ii. Approximately 1gram of the soil sample was taken in each of the nine test tubes. iii. 2-3 drops of prepared Saline solution were then added and mixed thoroughly by shaking. iv. A cotton thread was then taken and inserted so as the tip of the thread just touches the solution. v. This was then incubated overnight so as for the blood to soak through the thread by capillary action. vi. Each thread was then removed and a 2mm piece was cut placed on each cavity of a 12-cavity glass slide using DPX mountant. vii. Then one drop of antisera was added on basis of markings Anti-A was added in A and Anti-B was added in B respectively. viii. All sides were placed in a water dish lined with saline soaked filter paper (to keep the samples moistened at all times), ix. All slides were refrigerated at 4°C overnight. x. Next day to remove the excess antisera the slides were washed using chilled normal saline and repeated 3-4 times. xi. To this then blood cells A, B and O were added into the respective cavities. xii. This was then placed in the incubator at 56^0 Celsius for 20 minutes. xiii. Finally, light microscope is used for observation of agglutination, and same is repeated at the time interval of 5days for each sample. ivx. The same was repeated for each soil sample for a span of 20 days with a 5-day time interval.

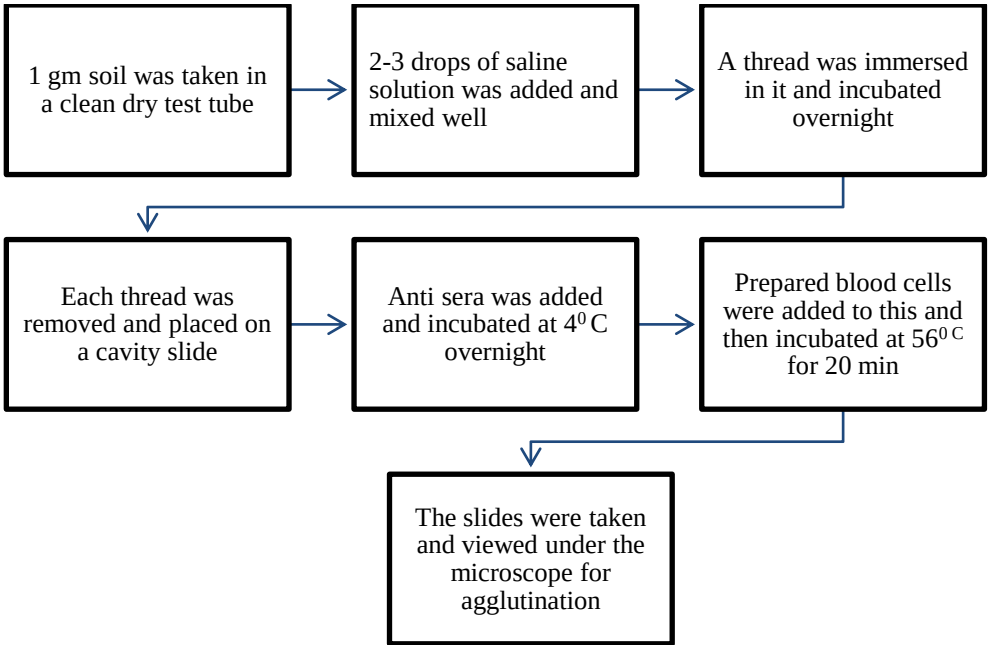

Figure-1: Flow chart explaining absorption elution procedure.

Figure-2: Each sample with thread inserted for cappillary action.

Figure-3: Each thread attached to the cavity slide with dpx mount.

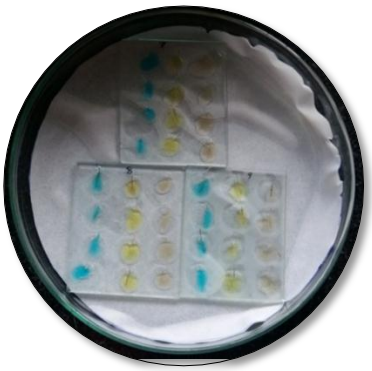

Figure-4: Appropriate anti sera added to each cavity.

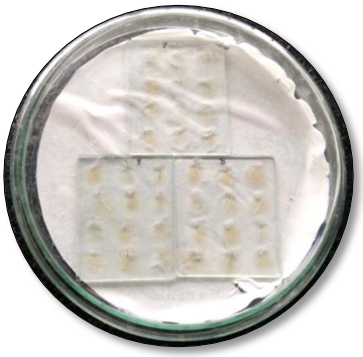

Figure-5: Samples for analysing under microscope to check for agglutination.

Results and discussion

Effect of Soil Temperature on Blood Grouping Examined by Absorption Elution Technique. Absorption elution technique is a method used to determine blood grouping for dried blood samples. Specific antibodies can absorb on the blood stain by keeping in the cold. They are then heated in the presence of indicator cells to elute the bound antibodies.

Table-1: The results from the absorption elution technique done for this study to analyse blood grouping are given. The same can be interpreted as below:

Microscopic agglutination
+++ : Very large agglutination clumps with few free cells.
++ : Smaller agglutinates with more free cells.
+ : Agglutinates of 5-10 cells with many free cells.
Neg : No agglutination

Effect of Temperature on Blood Grouping in Sea Side Soil Samples: Results: Agglutination results were noted and analysed for temperature on a 5 days span for 20 days. On the 5th day of blood grouping for room temperature i.e. 22^0 Celsius has smaller agglutination with more free cells were seen on the slides for all blood groups. On the 10th, 15^{th} and 20th day the results for all three blood groups were same as the 5th without any change in the agglutination. Very good agglutination results were seen on all the slides of A, B, O for cold temperature i.e. 5^0Celsius on all four days of analysis. For hot temperature i.e. 45^0 Celsius the agglutination results were smaller with more free cells on the 5th, 10th and 15th day for all three groups. No agglutination was observed for the 20th day on all the slides.

Discussion: Results indicate that most samples in these sample sets had resulted positive for blood grouping. This shows that exposing bloodstains to a naturally variable temperate climate such as room temperature and cold temperature can give positive results even on a span of 20 days. And when blood is exposed to heat for more than 15 days negative results are obtained for blood grouping in this particular soil. Table 4.7 also shows that maximum agglutination is seen in cold temperature, room and heat temperatures show a similar agglutination up to the 15th day. Hence from the above table it can be seen that, in Sea side soil, at room and cold temperature conditions, positive blood grouping can be observed even up to 20 days (i.e. 480 hrs). In heat temperature, the results had come negative on the 20th day (i.e. 480 hrs). This suggests that the blood degrades faster in heat compared to room and cold temperatures, and the negative results can imply that heat along with the contaminations from the soil might have affected the blood grouping results.

Table-2: Results of Absorption Elution for blood grouping test.

Soil Type	Temp	At 5 Days			At 10 Days			At 15 Days			At 20 Days		
		A	B	O	A	B	O	A	B	O	A	B	O
Sea Shore/ Sand	Room	++	++	++	++	++	++	++	++	++	++	++	++
	Cold	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Heat	++	++	++	++	++	++	++	++	++	Neg	Neg	Neg
Forest	Room	++	++	++	++	++	++	++	++	++	+	+	+
	Cold	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Heat	++	++	++	+	+	+	+	+	+	Neg	Neg	Neg
Agriculture	Room	++	++	++	++	++	++	Neg	Neg	Neg	Neg	Neg	Neg
	Cold	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++
	Heat	++	++	++	++	++	++	Neg	Neg	Neg	Neg	Neg	Neg
Mechanic Shop	Room	++	++	++	++	++	++	+	+	+	+	+	+
	Cold	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++
	Heat	++	++	++	+	+	+	Neg	Neg	Neg	Neg	Neg	Neg
Construction Site	Room	++	++	++	++	++	++	++	++	++	+	+	+
	Cold	++	++	++	++	++	++	++	++	++	++	++	++
	Heat	++	++	++	+	+	+	Neg	Neg	Neg	Neg	Neg	Neg

Results of Absorption Elution Technique for Forest Soil / Wet Land Samples: Results: A, B, O agglutination results were noted and analysed for each temperature on a 5 days span for 20 days. On the 5th, 10th and 15th day of blood grouping for room temperature i.e. 22^0 Celsius smaller agglutination with more free cells were seen on the slides for all three blood groups. On the 20th day the agglutination of 5-10 cells with many free cells were seen. Very good agglutination results were seen on all the slides of A, B, O for cold temperature i.e. 5^0 Celsius on all four days of analysis. For hot temperature i.e. 45^0 Celsius the agglutination results were smaller with more free cells on the 5th day and on the 10th and 15th day comparatively lesser agglutination was observed for all three groups. No agglutination was observed for the 20th day on all the slides.

Discussion: Results indicate that blood present at cold temperature shows maximum agglutination when compared to the rest. This shows that exposing bloodstains to a naturally variable temperate climate such as room temperature and cold temperature can give positive results even after a span of 20 days. And when blood is exposed to heat, the agglutination of blood decreases and becomes negative on the 20th day. Hence from the above table it can be seen that, at room and cold temperature conditions, in Forest soil, positive blood grouping can be observed even up to 20 days (i.e. 480 hrs). In heat temperature, the results had come negative after the 15th day (i.e. 360 hrs). This suggests that the blood degrades faster in heat compared to room and cold temperatures, and the negative results can imply that heat along with the contaminations from the soil might have affected the blood grouping results.

Results of Absorption Elution Technique for Agricultural Soil Samples: Results: A, B, O agglutination results were noted and analysed for each temperature on a 5 days span for 20 days. On the 5th, and 10th of blood grouping for room temperature i.e. 22^0 Celsius smaller agglutination with more free cells were seen on the slides for all three blood groups. On the 15th and 20th day no agglutination was observed on all the slides. Very good agglutination results were seen on all the slides of A, B, and O blood groups for cold temperature i.e. 5^0 Celsius on the 5th and 10th day. Comparatively smaller agglutination with more free cells was observed for the 15th and 20th day of analysis. For hot temperature i.e. 45^0 Celsius the agglutination results were smaller with more free cells on the 5th day and 10th and on the 15th and 20th day no agglutination were observed for all three blood groups.

Discussion: Results indicate that, in agriculture soil, exposing bloodstains to cold temperature shows maximum agglutination and best results when compared to the rest even after a span of 20 days. And when blood is exposed to heat or kept at room temperature, the rate of agglutination was less compared to cold and the results had become negative from 15th day. This may be due to the presence of microbes or other contaminants which may degrade the blood faster compared to other soils. Hence from the above table it can be seen that, only at cold temperature conditions, in Agriculture soil, positive blood

grouping can be observed even up to 20 days (i.e. 480 hrs). In room and heat temperature, the results had come negative from the 15th day (i.e. 360 hrs). This suggests that in this soil, the blood degrades faster in heat and room compared to cold temperatures, and the negative results can imply that heat along with other substances such as fertilizers, manure etc might have affected the blood grouping results.

Results of Absorption Elution Technique for Mechanical Shop Samples: Results: A, B, O agglutination results were noted and analysed for each temperature on a 5 days span for 20 days. On the 5th, and 10th of blood grouping for **room** temperature i.e. 22^0 Celsius smaller agglutination with more free cells were seen on the slides for all three blood groups. On the 15th and 20th day the agglutination of 5-10 cells with many free cells were observed on all the slides. Very good agglutination results were seen on all the slides of A, B, O for cold temperature i.e. 5^0 Celsius on the 5th and 10th day. Comparatively smaller agglutination with more free cells was observed for the 15th and 20th day consequently. For hot temperature i.e. 45^0 Celsius the agglutination results were smaller with more free cells on the 5th day and on 10th day smaller agglutination with 5-10 free cells were observed. On the 15th and 20th day no agglutination was observed for all three blood groups.

Discussion: Results indicate that as time progressed the rate of agglutination had decreased in all 3 temperature conditions. Bloodstains kept at cold and room environment had shown positive results even after a span of 20 days although the rate of agglutination had decreased as time progressed. But blood when exposed to heat, the results have been shown negative for ABO blood grouping from the 15th day. The table shows that bloodstains kept at cold temperature can show maximum agglutination and bloodstains kept exposed to heat shows the least after a span of 20 days. Hence from the above table it can be seen that, for Mechanical shop soil, at room and cold temperature conditions, positive blood grouping can be observed even up to 20 days (i.e. 480 hrs). In heat temperature, the results had come negative from the 15th day (i.e. 360 hrs). This suggests that the blood degrades faster in heat compared to room and cold temperatures, and the negative results can imply that heat along with the contaminations from the soil, such as the presence of oil, grease and other fuel contents might have affected the blood grouping results.

Results of Absorption Elution Technique for Construction Site Soil: Results: A, B, O agglutination results were noted and analysed for each temperature on a 5 days span for 20 days. On the 5th, 10th and 15th of blood grouping for room temperature i.e. 22^0 Celsius smaller agglutination with more free cells were seen on the slides for all three blood groups. On the 20th day the agglutination of 5-10 cells with many free cells were observed on all the slides. Smaller agglutination with more free cells was observed on all the slides of A, B, O for cold temperature i.e. 5^0 Celsius on all four days of analysis. Comparatively smaller agglutination with more free cells was observed for the 15th and 20th day consequently. For hot temperature i.e. 45^0 Celsius the agglutination results were smaller with more free cells on the 5th day smaller agglutination with more free cells was observed. On the 10th day the agglutination results were comparatively smaller with 5-10 cells. The 15th and 20th day showed no agglutination for all the three blood groups.

Discussion: Results indicate that most samples in these sample sets had resulted positive for blood grouping except for those exposed to heat. This shows that exposing bloodstains to a naturally variable temperate climate such as room temperature and cold temperature can give positive results even after a span of 20 days. And when blood is exposed to heat for more than 10 days negative results are obtained for blood grouping in this particular soil. Table-2 also shows that maximum agglutination is seen in cold temperature, and exposure to heat showed the least after a span of 20 days. Hence from the above table it can be seen that, in soil seen at construction site, at room and cold temperature conditions, positive blood grouping can be observed even up to 20 days (i.e. 480 hrs). In heat temperature, the results had come negative from the 15th day (i.e. 360 hrs). This suggests that the blood degrades faster in heat compared to room and cold temperatures, and the negative results can imply that heat along with the contaminations from the soil might have affected the blood grouping results.

Conclusion

The present study can be concluded that examination of blood itself is a vast task and when blood is contaminated with soil it even harder. Since, soil is also important evidence which is commonly found in all crime scenes hence, blood-stained soil is not an unexpected. Soil can provide significant information in investigation as transfer of soil and blood can be encountered when crime takes place in the outdoor. Thus, soil can also be a good source of evidence in violent cases particularly with blood stain. The present study purposes are to explore the importance of blood contaminated with soilas forensic evidence. The comparison of soils validates that different types of soils have shown the ability to retain blood in its original nature, effect of soils on purity and degradation of blood and effect of temperature in retaining the evidentiary value in the blood to identify and to analyse to individualise the culprit and victim of the crime, by which the very role of forensic will be fulfilled.

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