# EXPLORING THE INTERACTION OF BLOOD AND SOIL

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**Abstract**

The study involved the observation of changes in blood mixed with soil samples over a period of 10 days, with an interval time of 2 days. It involved the collection of five soil samples from the crime scene, where blood was found mixed with the soil. The soil samples were subjected to four tests, namely, pH test, particle size distribution test, loss of ignition test, and density gradient test. The aim was to investigate the degradation and decomposition of blood and its interaction with soil. The results of the study are expected to provide valuable insights into the potential changes that may occur in blood when mixed with soil, and how these changes can be used to help in forensic investigations. The findings of this study may also have important implications for the field of forensic science, as they may help in the development of new techniques and methods for analysing soil samples in criminal investigations.

**Keywords –**

Blood, Soil, pH test, Particle Size Distribution Test, Loss on Ignition Test, Density Gradient Test.

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**Introduction –**

Forensic science is a multidisciplinary field that uses scientific methods to investigate and resolve criminal cases. An important aspect of forensic science is the analysis of soil samples taken from the crime scene. Land analysis can provide important information about the location and movement of victims, victims or crime objects. Especially the presence of blood in soil samples can provide important evidence in cases involving crime or murder.

Analysing the blood-soil mixture is a difficult task due to the complexity of the soil and the difference in blood composition. Blood is a complex biological fluid containing many organic and inorganic compounds, including proteins, lipids, carbohydrates and ions. Soil is a mixture of minerals, organic matter, water and air. Soil properties can vary depending on soil type, location, and history. In this study, we focus on the analysis of blood-dirt mixture collected from different locations in Ratlam, Madhya Pradesh, India.

Our aim is to examine changes in the blood-soil mixture using a combination of physical and chemical tests. More specifically, we performed the pH test of the mixture, the particle size distribution test, the misfire test, and the density gradient test on the soil and also the preliminary tetra methyl benzidine (TMB) test for examination of blood. We also observed changes in the blood-soil mixture over 10 days. The results of this study may provide a better understanding of the behaviour of blood in soil and help researchers analyse criminal evidence. By understanding how the blood dirt mixture changes over time, researchers can determine when blood is left behind and the movement of the suspect or victim. This information can be vital to solving crimes and ensuring justice for victims and their families.

**Review of Literature –**

**Laura Giacomelli, Silvia Armienti, and Claudia Cappella (2020)** - This paper describes a method for the analysis of soils collected from crime scenes to identify organic compounds, such as blood or other bodily fluids, using solid-phase micro extraction and gas chromatography-mass spectrometry. The authors demonstrate that this method can successfully identify compounds from soil samples that were artificially spiked with blood.

**Francesco Buscemi, Cinzia Fabbri, Annalisa Alù, Francesco Introna, and Daniele Gibelli (2018)** - This paper discuss the effect of temperature and humidity on the recovery of DNA from soil samples that were artificially spiked with blood. The authors found that higher temperatures and humidity levels reduced the amount and quality of DNA that could be recovered from the samples.

**Ana I. Martinez-Perez, Maria-Josep Iglesias, Juan A. Riquelme-Cabello, and Inmaculada Ortega-Mora (2019)** - This paper investigates the stable isotopic composition of human bone collagen in different soil types, with the goal of understanding how soil type can impact isotopic analyses for forensic purposes. The authors found that the isotopic composition of bone collagen was affected by the surrounding soil, but that this effect varied depending on the soil type.

**Emma Wilkins, Michael W. Halpenny, Gregory M. Miller, and Lorna A. Dawson (2021)** - This paper presents a method for the analysis of soils using high-resolution mass spectrometry to identify and quantify trace amounts of organic compounds, such as blood or other bodily fluids. The authors demonstrate that this method can successfully identify compounds from soil samples that were artificially spiked with blood.

**Davide Mazzoni, Francesca Magi, and Daniele Nani (2019)** - This paper discuss the potential of microscopic analysis of soils for forensic purposes, focusing on the identification of bloodstains in soil samples. The authors found that microscopic analysis can provide valuable information about the 18 locations, age, and origin of bloodstains in soil samples, which can help to link a crime scene to suspect.

**M. Benitez-Navarro, F. Martinez-Garcia, J. M. Almendros, and J. A. Zazo (2016)** - This paper explore the effect of soil type and moisture content on the preservation of bloodstains in soil, with the goal of improving the reliability of forensic analyses. The authors found that bloodstains tended to be better preserved in well-drained, sandy soils with low organic matter content.

**K. V. Rossmo, K. B. Maloney, and A. R. Bucholtz (2018)** - This paper discusses the use of geographic profiling to investigate cases involving the interaction of blood and soil. The authors present case studies in which geographic profiling was used to identify potential suspects based on the location of crime scenes and the distribution of soil types.

**S. T. Crawford, S. S. Eshelman, and K. L. Koenig (2018)** - This paper describes a method for the analysis of soil samples collected from crime scenes to identify bloodstains using visible and near infrared reflectance spectroscopy. The authors demonstrate that this method can accurately detect bloodstains in a variety of soil types, including those with high levels of organic matter.

**M. R. Silwood, D. D. Mills, S. D. Ross, and P. R. Sutton (2020)** - This paper investigate the chemical interactions between blood and different types of soil using Fourier transform infrared (FTIR) spectroscopy. The authors found that the chemical properties of the soil had a significant impact on the interactions between blood and soil, with clay soils exhibiting the strongest interactions.

**Material and Methodology –**

**Sample Collection:** We collected five soil samples from different locations in Ratlam city in Madhya Pradesh, India. The samples were collected using a sterile spatula and placed in individual sterile containers. The samples were labelled.

1. Pot Soil

2. Lake Soil

3. Roadside Soil

4. Field Soil (Black)

5. Garden Soil

**Preparation of Blood-Soil Mixture**: To prepare the blood-soil mixture, we used fresh blood from a healthy donor. We mixed 5 mL of blood with 50 g of each soil sample, using a sterile spatula. We repeated this process for all five soil samples to create five blood-soil mixtures. We then allowed the mixtures to settle for 24 hours to ensure uniform mixing.

**pH Test:** pH paper was used to measure the pH of the blood-soil mixture. pH paper is a simple and inexpensive tool that can provide a quick estimate of the pH value of a solution. To perform the pH test, a small piece of pH paper was dipped into the blood-soil mixture and the colour change of the paper was compared to a pH colour chart. The pH value of the mixture was then determined based on the colour match of the pH paper with the chart.

**Particle size Distribution:** Particle size distribution (PSD) test is a method used to determine the proportion of different sized particles in a soil sample. Soil particles are classified into three main categories based on their size: sand (larger than 2.0 mm), silt (0.002-0.02 mm), and clay (smaller than 0.002 mm). The PSD test measures the percentage of each particle size in the soil sample. To conduct the PSD test, a soil sample is first air dried, and then passed through a series of sieves with different sized openings. The soil is shaken for a specified period of time, and the material that remains on each sieve is weighed. The weight of each size fraction is used to calculate the percentage of each particle size in the soil sample.

**Loss of Ignition Test:** To perform the loss of ignition test, we weighed 10 g of the blood-soil mixture and placed it in a crucible. We then heated the crucible to 550°C for 2 hours in a muffle furnace. We then weighed the crucible and recorded the weight loss of the mixture.

**Density Gradient Test:** We created a density gradient column by layering 10 mL of bromo-benzene and 10 mL of xylene in a test tube. We then carefully added 1 mL of the blood-soil mixture on top of the density gradient using a pipette. The density gradient test allows the separation of components based on their density. As the mixture settles, the components of different densities will move through the column and form distinct bands. We observed the bands formed by the different components and recorded the results.

**Tetra Methyl Benzidine (TMB) Test:** An acetate buffer solution is prepared first by adding 5g of sodium acetate in 13ml glacial acetic acid and 57ml distilled water. Afterwards a working solution is prepared by mixing 1.5g of tetra methyl benzidine (TMB) solution in 20ml of acetate buffer solution. To test the presence of blood in the soil, a portion of soil is taken on filter paper and then 4-5 drops of working solution is added to the soil along with the addition of few drops of hydrogen peroxide solution and the change of colour on the filter paper is seen. If the paper has stains of bluish green colour then it confirms the presence of blood in the soil.

**Observation and Result –**

**pH Test –** The resultants of pH examination reveals the fact that day by day the interaction of the blood with the soil increases its basicity up to a frequent point and further on basic nature starts to decrease because of the contamination of the blood. This gradual increase and further decrease tells that soil and blood have interacted with each other for few days.

**Table 1 pH Examination**

| Sample No. | Day 1 | Day 4 | Day 7 | Day 10 |
|---|---|---|---|---|
| **1** | 7 | 9-10 | 9-10 | 9 |
| **2** | 8 | 8-9 | 9-10 | 8 |
| **3** | 7 | 8-9 | 10-12 | 8-9 |
| **4** | 7 | 7-8 | 10-12 | 9-10 |
| **5** | 7 | 8-9 | 12-14 | 8-9 |

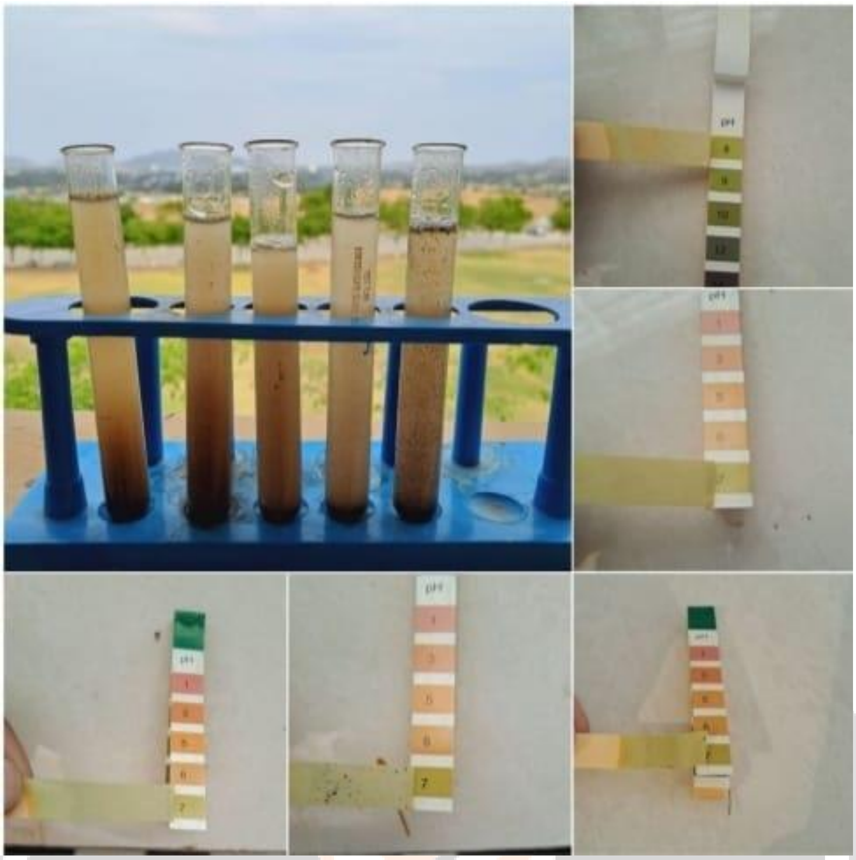

Figure 1 pH examination of blood soil complex day 1

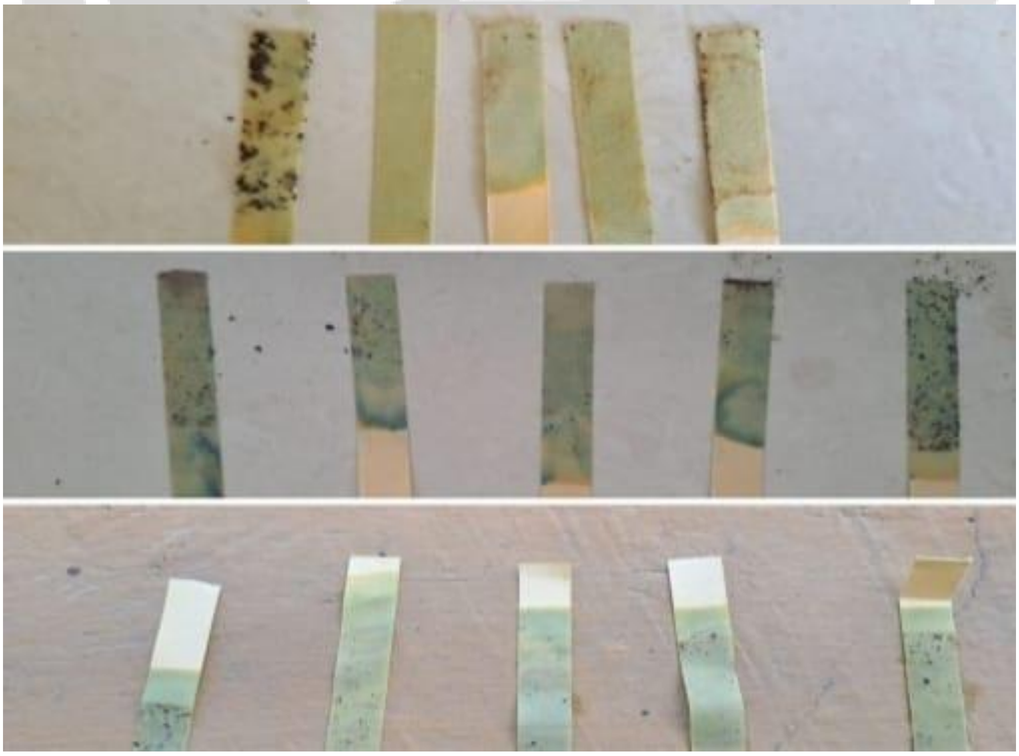

Figure 2 pH examination day 4, day 7 and day 10

**Particle Size Distribution Test –** The different granule size particles of the soil of five different areas are distributed using sieves of different particle sizes.

Figure 3 Particle Size Distribution of the five different types of soil fetched from five different places

**Loss-on Ignition Test –** The gradual decrease in weight of the soil-blood interactive complex after every testing basically tells us that as the day passes the different compounds which are present in the complex vaporises from the complex by losing its bonds and hence the volatile substances leaves the complex.

Table 2 Loss-on Ignition on Day 1

| Weight of Soil before Ignition | Weight Loss after Ignition |
|---|---|
| 5.6g | 5.3g |
| 5.1g | 4.8g |
| 6.8g | 6.2g |
| 5.5g | 5.1g |
| 3.2g | 2.8g |

Figure 4 Loss-on ignition test day 1

Table 3 Loss-on Ignition on Day 4

| Weight of Soil before Ignition | Weight Loss after Ignition |
|---|---|
| 5.3g | 5.1g |
| 4.8g | 4.2g |
| 6.2g | 5.2g |
| 5.1g | 4.3g |
| 2.8g | 2.1g |

Figure 5 Loss-on ignition test day 4

Table 4 Loss-on Ignition on Day 7

| Weight of Soil before Ignition | Weight Loss after Ignition |
|---|---|
| 5.1g | 4.4g |
| 4.2g | 3.7g |
| 5.2g | 4.3g |
| 4.3g | 3.7g |
| 2.1g | 1.9g |

Figure 6 Loss-on ignition test day 7

**Table 5 Loss-on Ignition on Day 10**

| Weight of Soil before Ignition | Weight Loss after Ignition |
|---|---|
| 4.4g | 3.7g |
| 3.7g | 3.3g |
| 4.3g | 3.9g |
| 3.7g | 3.2g |
| 1.9g | 1.1g |

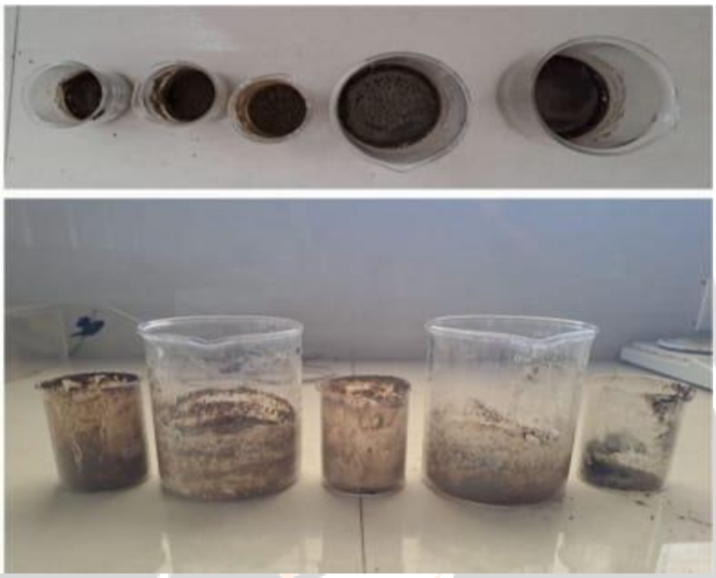

**Figure 7 Loss-on ignition test day 10**

**Density Gradient Test –** Soil of different area have given different layers of soil particles on the density column as each type of soil have separate density and due to addition of blood and interaction of soil and blood, there were some minute changes in the density of that complex and due to addition of blood, the blood got mixed with the solvent system as a result of which the solvent appears reddish-brown in colour.

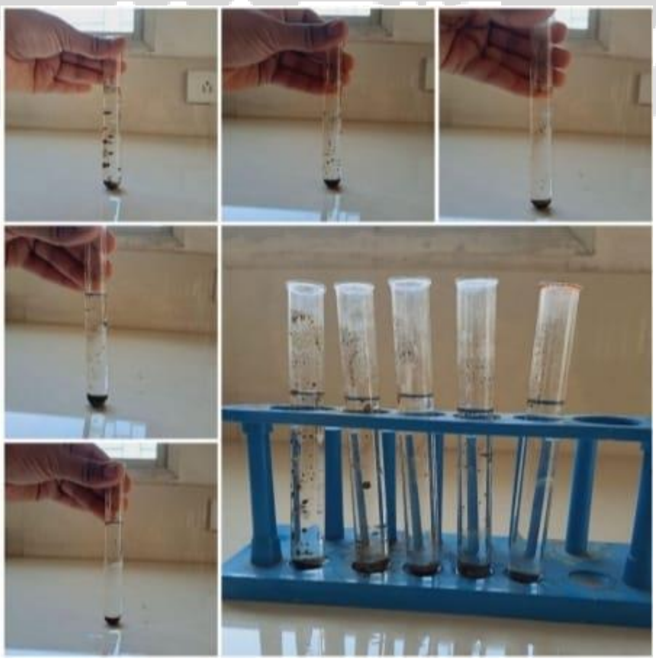

**Figure 8 Density Gradient Test on the Blood Soil Complex**

**Tetra Methyl Benzidine (TMB) Test –** The blood gave positive test up to 4 days but afterwards might be due to contamination of the blood in the blood soil complex there was no change of colour on the paper.

**Table 6 TMB Test on the Blood-Soil Complex**

| Days of Examination | TMB test resultant on blood |
|---|---|
| Day 1 | Positive (Bluish-Green Colour) |
| Day 4 | Positive (Bluish-Green Colour) |
| Day 7 | Negative (No Change in Colour) |
| Day 10 | Negative (No Change in Colour) |

**Conclusion –**

To observe changes in the blood-soil mixture over time, we stored the mixtures in a controlled environment at room temperature. We then recorded changes in the pH, particle size distribution, loss of ignition, and density gradient over the next 10 days at 1st, 4th, 7th and then 10th day. All the changes are recorded properly. Yes, the minute changes are observed on the blood-soil complex after the passage of each and every subsequent day.

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