Tec Empresarial
P-ISSN: 1659-2395; E-ISSN: 1659-3359

COMPARATIVE STUDIES OF DEGRADATION OF BIOLOGICAL FLUIDS OF FORENSIC IMPORTANCE ON DIFFERENT SURFACES AT DIFFERENT TEMPERATURE AND TIME INTERVALS

[1]Neetu Sharma, [2]Jaskaran Singh
[1]Research Scholar, School of Sciences, Geeta University, Panipat, India
[2] Professor, School of Sciences Geeta University, Panipat, India
Email: {msneetumehta, jaskaransingh630 } @gmail.com

Corresponding Author: Jaskaran Singh

1. ABSTRACT

Blood and other body fluids collected at crime scenes are sensitive to the surrounding environmental contamination and growth of microorganisms which can destroy their evidential value before damage to them can be assessed in the laboratory. This deterioration can depend on a number of environmental conditions, including temperature, duration of exposure, surface nature on which the biological material rests. The understanding of these factors is critical for the best evidence collection strategy, and for maintaining the integrity of biological samples to be used for forensic analysis.

The purpose of the present study is to investigate the microbial breakdown of two biological fluids present in the environment: blood and saliva, that were placed on five different inanimate surfaces (soil, glass, wall, floor and ceramic tile) and with two different environmental temperatures (4-9°C, 20-25°C and 40-45°C). Healthy volunteers' 5 blood samples & 5 saliva samples were then exposed for 6, 12, 18, 24 & 48 hours. Conventional culture methods, staining techniques, selective media and standard biochemical tests were used to identify the microorganisms associated with each sample to assess microbial colonization and progression of biological fluid degradation.

It was found that both growth of microbes and disruption of biological fluids highly depended upon the environmental temperatures and surface characteristics. Soil always had the highest level of biological fluid degradation and the highest microbial diversity of all the substrates evaluated. Good deterioration was observed in the samples at 20–25°C, with the highest levels between 12 and 24 hours followed by growth of microorganisms at 40-45^C. At 4–9°C, on the other hand, several non-porous surfaces, such as glass, wall surfaces and ceramic tiles, showed very little or no detectable bacterial growth after 12 hours. Progressive microbial activity in saliva correlated with a decrease in detectable amylase activity and hemolytic *Streptococcus* species were found to be significant blood degraders, reducing amylase's forensic value.

All of these indicate that environmental temperature, time of exposure, and substrate can act complementary in the preservation of biological fluids at a crime scene. The findings highlight the fact that efficient evidence recovery and preservation practices are critical for successful forensic laboratory handling of these items to ensure proof value and minimize microbial impacts, especially from ambient environments.

Keywords: Biological fluids; forensic evidence; microbial degradation; blood; saliva; environmental temperature; crime scene investigation.

2.

3. INTRODUCTION

Most of biological evidence found at crime scenes plays a significant part in the forensic investigation and plays a vital role in the process of criminal investigation, including personal identification, reconstruction of criminal incidents (Aloraer, 2017), establishing connections between suspected person(s), victim(s), and the scene(Aloría, 2016). However, blood and saliva are more often seen in different type of biological evidence as it is likely to be deposited in violent offences, assaults, burglars and other criminal incidents (Upadhyay et al., 2023). Ultimately, the forensic significance and utility of these biological fluids depends on how they have been preserved from the time of their deposition to the time of examination in the laboratory, and the forensic value decreases over time as they are exposed to the environment and their evidential integrity diminishes(Zapata et al., 2014).

Preservation of cellular and biochemical components of blood and saliva are of close association with the evidential significance. Plasma in blood and cellular components, proteins, enzymes, and microorganisms present in the blood, while cellular components in saliva as well as proteins, enzymes, and microorganism present in the oral cavity. (Song et al., 2014; Upadhyay et al., 2023) These constituents can be important for information to DNA typing, body fluid identification and serological analysis or other forensic examinations. At a crime scene however, biological fluids are subjected to physical, chemical and biological alterations that will compromise an analysis from the time they are deposited forward into the environment.

Under natural environmental conditions, microbial contamination is one of the major mechanism causing deterioration of biological fluids(Ogdur et al., 2018). Proteins, carbohydrates, lipids and other organic components are all used by environmental bacteria and fungi as well as biological sample's inherent microorganisms as the sources of nutrients(Lim et al., 2005). As the number of days goes up, their metabolism gradually damages the cell structure, compromises the enzyme and biochemical activity, and leads to nucleic acid degradation resulting in less reliable forensic processing. Presumptive tests, confirmatory examinations, DNA profiling and interpretation of biological evidence may be affected by degradation, with the accuracy being reduced as degradation progresses (Pajnič, 2025).

Microbial degradation constitutes the condition degradation as it is considered to be one of the most influential factors to microbial decomposition rate since it directly influences microbial metabolism, growth and survival processes (Iancu et al., 2024). Lower temperatures are generally bacteriostatic thus are disadvantageous in terms of the multiplication of many microorganisms and moderate temperatures good in favor of the rapid multiplication of microorganisms and accelerate biological degradation (Bisker et al., 2021). Under these circumstances, if biological evidence is left unpreserved in the surrounding Environments, it can very rapidly become significantly affected.

Porous and non-porous substrates vary in their ability to absorb moisture, organic matter and microorganisms(Nasir et al., 2026; Ogdur et al., 2018). For example, the microbial biomass present in soil is able to rapidly colonize biological fluids, while relatively smooth, non-porous, surfaces like glass, ceramic tile and painted walls typically offer less favorable conditions for the maintenance of microbial presence in biological fluids (Verdier et al.,

2014). The change in moisture, aeration and nutrient availability of these substrates may end up affecting microbial diversity and the degradation of biological evidence.

The nature of biological fluids in the hours following their deposition is another important biological determinant of degradation (Nasir et al. 2026; Salzmann et al. 2021). Over time, however, microorganisms increase in number and begin to eat away at the nutritious substances and gradually change the physical and biochemical aspects of the stain (Divya et al., 2024). As a result, evidence deteriorates when there is delay in evidence recovery which concretely affects the results of the lab investigation, and subsequently leads to poor or incomplete decipher and/or successful analysis outcomes(Swayambhu et al., 2023). Being familiar with the relationship between exposure and associated microbial colonizing is therefore crucial to formulating best practices to optimize microbial sample preservation methods.

While some factors have been investigated for their effect on the degradation of the DNA in biological evidence, most previous studies have focused on DNA degradation, single environmental factors, or single biological fluids under laboratory conditions with controlled environment conditions(Divya et al., 2024; Elliott et al., 2022). The combined influence of both surface type and the time of exposure with temperature on the microbial colonization of blood and saliva is very little investigated. This information is of special relevance to forensic applications where biological evidence may be left behind for a variety of environmental conditions before it can be collected and sent to the laboratory for analysis.

The present study was designed to examine the microbial degradation of blood and saliva which were deposited on inanimate surfaces soil, glass, wall, floor and ceramic tile at exposure times of 6-48 hours in two different temperature ranges 4-9°C, 20-25°C and 40-45°C. Biological fluid associated microorganisms were isolated and identified by the conventional microbiological methods to get a grip on the effect of temperature, surface properties, and exposure time on the deterioration of the biological fluids. The results confirm the environmental stability of common forensic biological fluids and may be useful for forensic practitioners who wish to collect, preserve and examine evidence during forensic investigations.

4. MATERIALS, AND METHODS

4.2 Study Design

An experimental study was performed to study the effects of the environment temperature, surface and time of exposure on the microbial degradation of biological fluids of forensic importance. Preservation of the blood and saliva on various flat, inanimate surfaces when exposed to a controlled environment was investigated to determine the potential to preserve blood and the suitability of some surfaces for further forensic examination of saliva.

4.3 Biological Samples

The whole blood and saliva were chosen for the study since they are the two fluids that are commonly found in a crime scene(Falweisen et al., 2009; Sipsey et al., 2009). A total of five blood samples and five oral fluid (saliva) samples were taken from healthy adult volunteers in accordance with standard aseptic techniques. All specimens were immediately put into cold storage (<4 C) prior to experimental processing to reduce pre-analytical microbial contamination and preserve the integrity of the specimen until analysis.

4.4 Ethical Approval

Research protocol was reviewed and approved by the Institutional Ethics Committee (IEC)Vide Letter No.: IEC/ISFCP/2026/01/09. Only healthy adult volunteers gave informed consent either by writing for this study, where they provided whole blood and saliva samples. Participants were informed of the study's purpose, procedures for collecting the samples, hazards and benefits, and that they can leave the study at any time without repercussions or any damage. All participation was voluntary.

All biological sample materials underwent microbiological analysis using unique identification code to protect the privacy and confidentiality of the participants. None of the data from this study were recorded, stored or disclosed at any point in time which identified the subject. Bio-samples and related research data collected were only utilized for research purpose and they were managed according to ethical and confidentiality standards.

4.5 Experimental Design

The experiment protocol was developed to assess the synergistic effect of biological fluid type, environmental temperature, surface substrate and time of exposure on microbial degradation of biological fluid and colonization. Blood samples and saliva samples were put on five inanimate surfaces that are commonly found in the house (soil, glass, wall, floor and ceramic tiles) separately.

The surfaces tested were kept under two experimental temperatures:

- **Low-temperature Range:** 4–9°C
- **Ambient-temperature Range:** 20–25°C
- **High -temperature range:**40-45°C

The biological fluids were exposed for five predetermined times of sampling, of 6, 12, 18, 24 and 48 hours, for each temperature condition. Thus, the experimental design consisted of two fluids, three temperature ranges, five surface substrates, and five exposure times. In all there were 150 experimental samples selected for the study.

4.6 Sample Collection Following Environmental Exposure

The biological samples were then left undisturbed following deposition onto the surfaces being tested until after the exposure period was completed. Aseptically, samples were taken at each data collection point (6, 12, 18, 24, and 48 hours) with sterile cotton swab applicators.

This was to ensure that no cross-contamination would occur, using a different sterile swab each time.

The specimens were immediately taken for microbiological analysis after collection. Hence, there was a uniformity in the experimental procedure by applying the same sampling protocol for all surface types, exposure times and temperatures.

4.7 Table 1: Summary of Sampling:

SAMPLE (Biological fluid)	TEMPERATURE	SURFACE	TIME INTERVAL

Blood Saliva	4-9^C 20-25^C 40-45^C	Soil	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
	4-9^C 20-25^C 40-45^C	Glass	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
	4-9^C 20-25^C 40-45^C	Wall	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
	4-9^C 20-25^C 40-45^C	Floor	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
	4-9^C 20-25^C 40-45^C	Tile	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours

Isolation of Microorganisms

The recovered samples were placed on suitable microbiological culture media for the isolation of bacterial and fungal organisms. Bacteria culture and fungal isolation was carried out on nutrient agar and Sabouraud's dextrose agar respectively. All the media inoculated were cultured aerobically following standard microbiological laboratory procedures.

Soil samples were taken and microorganisms were isolated using serial dilution technique. Suspensions for soil were made with 0.85% sodium chloride solution and were serially diluted at log ten dilutions from 10^1 to 10^7. 100 ul aliquots of each dilution were spread on the Nutrient agar and Sabouraud's dextrose agar. After incubation, some typical colonies of bacteria and fungi were picked for further identification and characterisation.

4.8 Identification of Microorganisms

Conventional staining techniques, microscopic examination and biochemical characterizations were all used for the purpose of microbial identification. All bacterial isolates were gram stained and fungal were examined by Lactophenol Cotton Blue (LPCB) staining. If appropriate, motility testing was undertaken to aid in the identification of the bacterial species.

A series of standard laboratory assays was carried out to facilitate biochemical characterization with analyses such as:

- Indole
- Methyl Red
- Voges–Proskauer
- Citrate utilization
- Urease
- Nitrate reduction
- Catalase
- Coagulase
- Triple Sugar Iron (TSI) Agar
- Oxidase tests.

Selected isolates were further cultured on various selective and differential media: Blood agar, Chocolate agar, MRS agar, Mannitol Salt agar, Starch agar, MacConkey agar and CHROM agar, to confirm the microorganism's identity.

4.9 Assessment of Biological Fluid Degradation

The degree of microbial colonization was recorded for all biological fluids for all surface types, temperatures and exposure periods. Variances in microbial activity were then compared to determine the evolution of the biological fluid degradation processes, as well as the effect that environmental parameters have on the preservation of forensic evidence.

5. RESULTS

The process of microbial degradation of biological fluids under the low temperature (4-9°C) was assessed.

5.2 Blood Samples

Blood specimens obtained from all the experimental surfaces were mainly populated with the *Gram-Positive microorganisms* at the temperature of the experimental condition at 4-9°C. The bacterial diversity in soil was highest during the entire exposure period, with recovery of both Gram-negative and Gram-positive bacteria. The comparatively small microbial growth on glass, wall and tile surfaces was limited to no detectable bacteria after 12 hours. Gram-positive spore-forming bacilli and cocci were recovered only at a 6-hour interval on a glass surface, while Gram-positive cocci were recovered up to 12 hours on the glass and surface from the floor and thereafter, no further recovery of bacteria was noted.

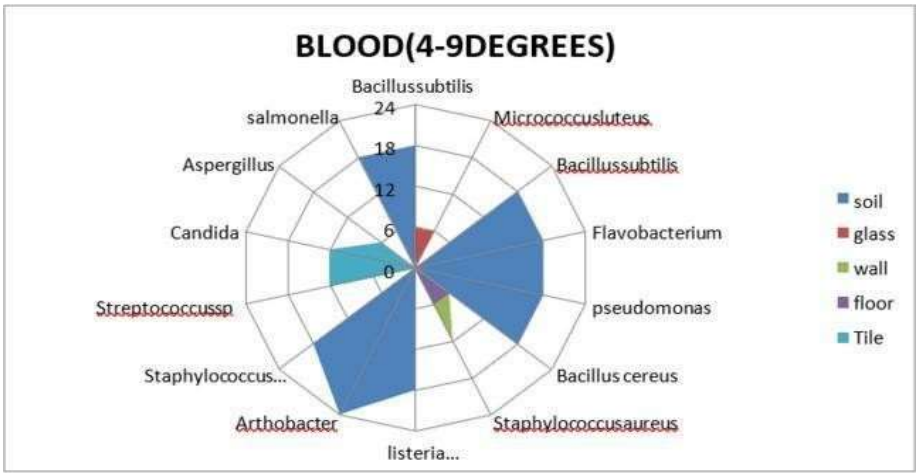


Figure 1. Distribution of *microorganisms* isolated from *blood samples* maintained at 4–9°C across different surfaces and exposure periods.

5.3 Saliva Samples

The growth of microbes in saliva samples was relatively small at lower temperatures (4-9°C). The presence of the fungi on the tile surface was identified after 6 hours of environmental exposure of Aspergillus species, but after 12 hours of exposure, the Candida species remained. Greater fungal survival in environmental exposure under refrigerated conditions was observed.

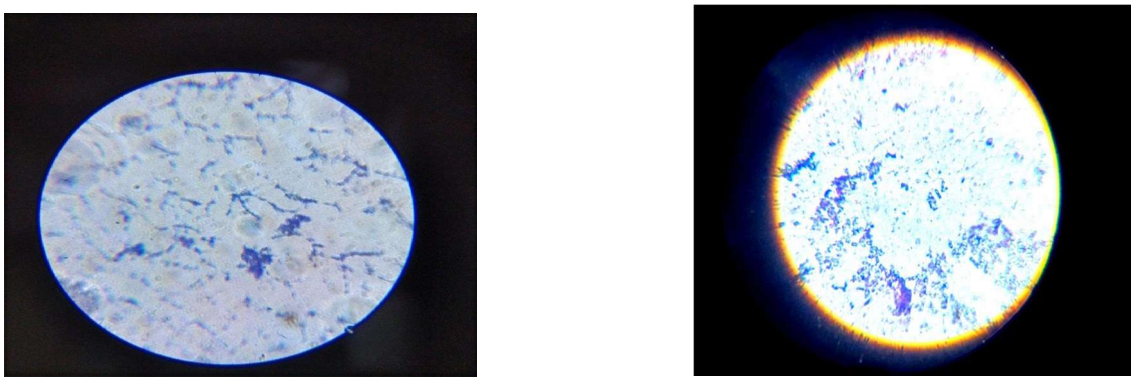


Figure 2. Representative isolates of *Bacillus cereus* and *Bacillus subtilis* recovered from blood samples maintained at 4–9°C.

On the glass surface, *Bacillus cereus* was isolated at the 6-hour time interval which was replaced by *Bacillus subtilis* after 12 hours, suggesting a shift in the dominant species of bacteria in environmental exposure over time.

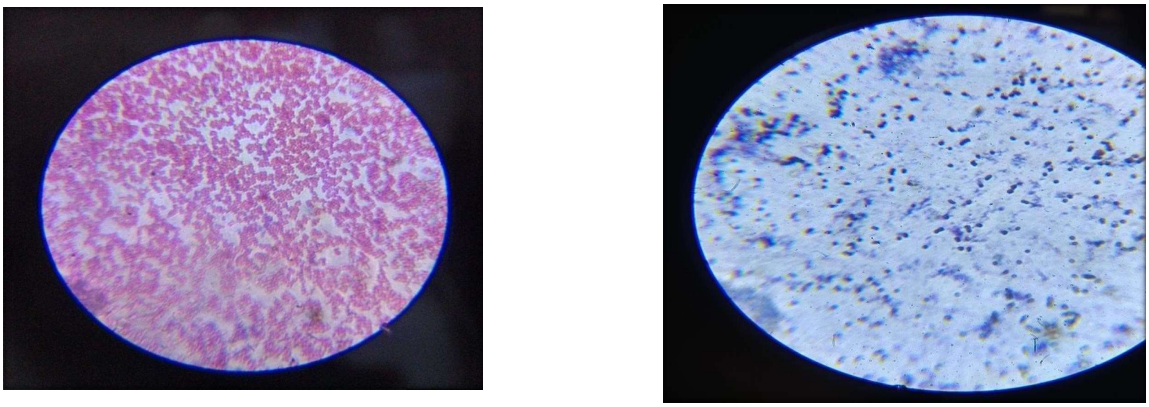


Figure 3. Representative isolates of *Pseudomonas aeruginosa* and *Lactobacillus* spp. recovered from saliva samples maintained at 4–9°C.

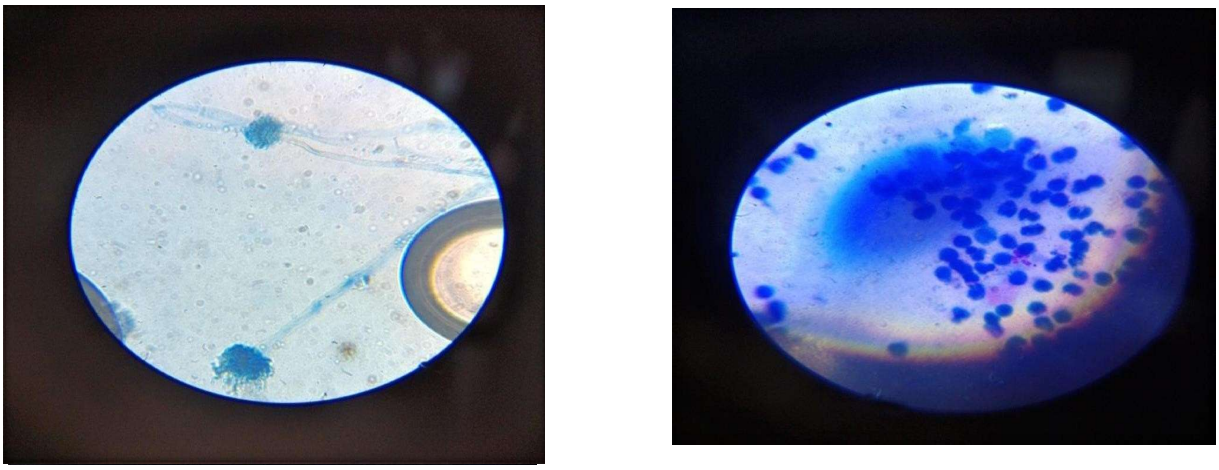


Figure 4. Representative isolates of *Aspergillus niger* and *Candida albicans* recovered from saliva samples maintained at 4–9°C.

A longer time was found for the detection of Pseudomonas and Lactobacillus species in the soil sample, with organisms being detectable up to 18 hours and 24 hours' environmental exposure, respectively.

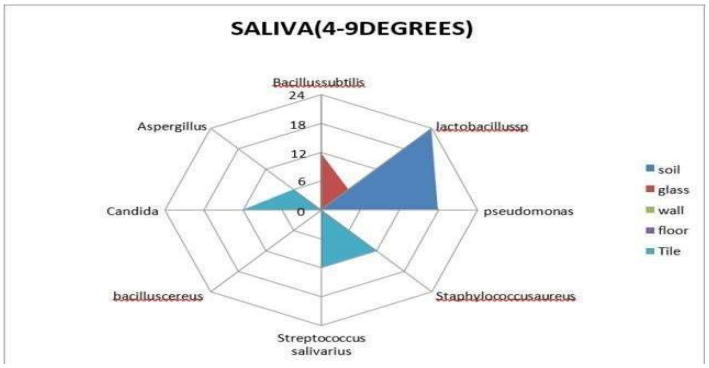


Figure 5. Distribution of *microorganisms* isolated from *saliva samples* maintained at 4–9°C across different surfaces and exposure periods.

Effect of Ambient Temperature (20–25°C) On Biological Fluids

Figure 6. Culture Plates isolated from blood samples maintained at 20–25°C across different surfaces and exposure periods.

- *Blood Samples*

The growth of microorganism was found to be significantly increased at T-24 h compared to T-12 h on most of the experimental surfaces under 20–25 °C. However, across the entire observation period, soil showed the highest level of microbial diversity, a greater number of microorganisms than was found in the other substrates that were tested (Figure 4).

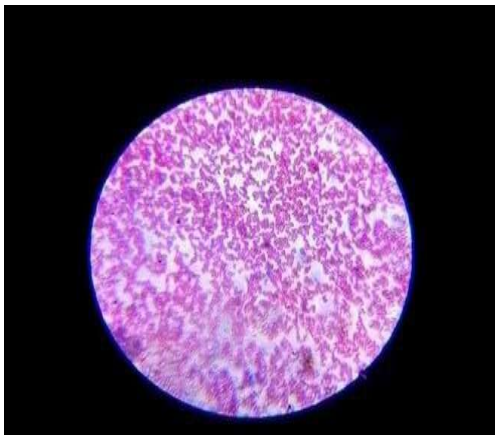
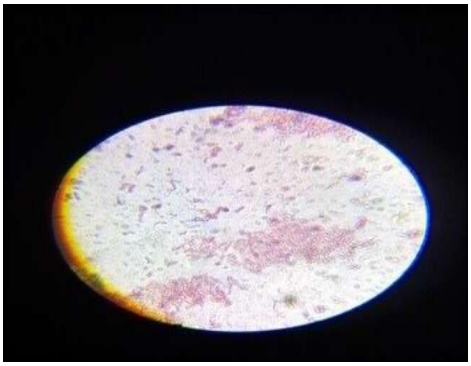


Figure 7. Representative isolate of *Salmonella* recovered from blood samples maintained at 20–25°C.

There was a temporal change in the bacterial population of the environmental samples with the early hours being gram-negative cocci and bacilli, and the later months exhibiting increasing numbers of the gram-positive rods and cocci.



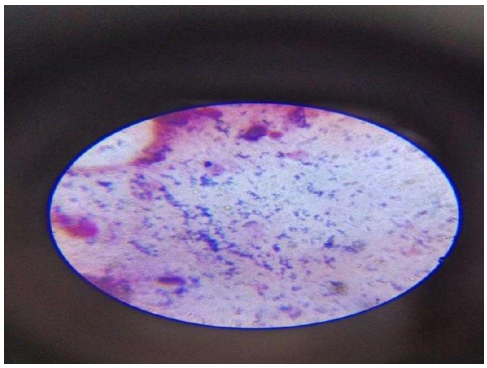


Figure 8. Representative isolates of *Escherichia coli* and *Bacillus spp.* recovered from blood

The wall surface did not have any detected micro growth after 24 hours of exposures. In the microorganisms isolated from blood samples, hemolytic *Streptococcus* species were one of the major isolates and were often seen in advanced stage of blood degradation.

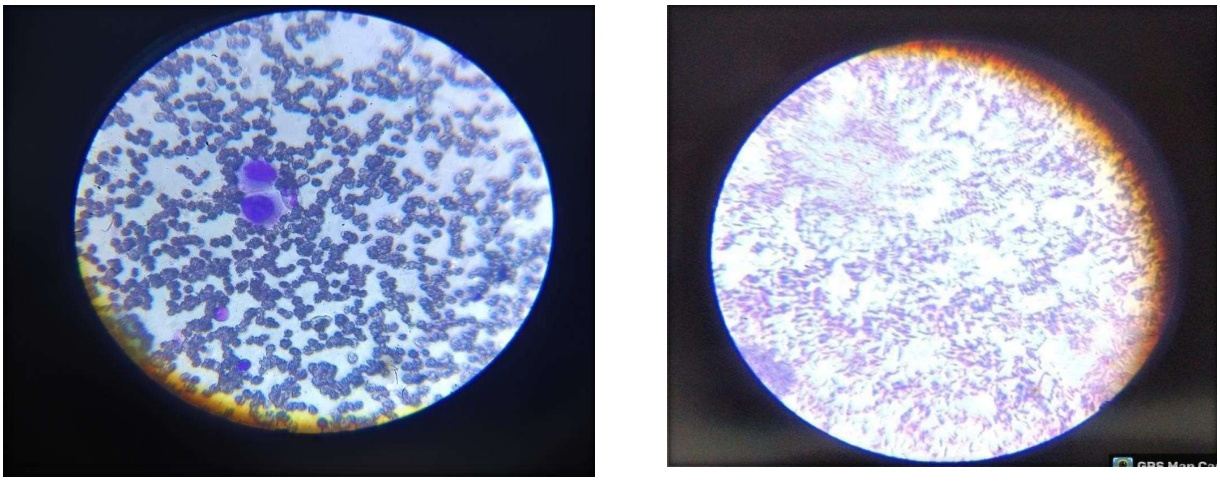


Figure 9. Representative Gram-positive cocci (*Staphylococcus aureus* and *Streptococcus* spp.) isolated from blood samples maintained at 20–25°C

After 18 hours of exposure, samples collected from the floor, tile, and glass surfaces with multi-component bacterial and fungal communities were found, suggesting continued microbial colonization during the conditions present in the room.

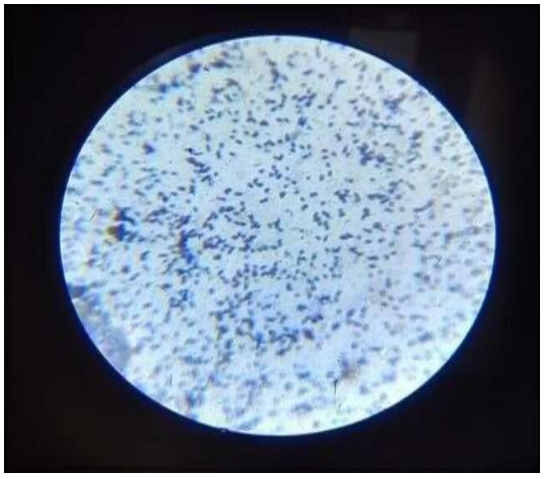


Figure 10. Photomicrograph of *Streptococcus pneumoniae* isolated from *blood samples* maintained at 20–25°C

The glass, wall, floor and tile surfaces had reduction in microbial recovery at the 24-hour exposure interval. However, the number of Bacillus cereus was a major part of the glass surface, which could not be ignored, because the microbial recovery level decreased overall.

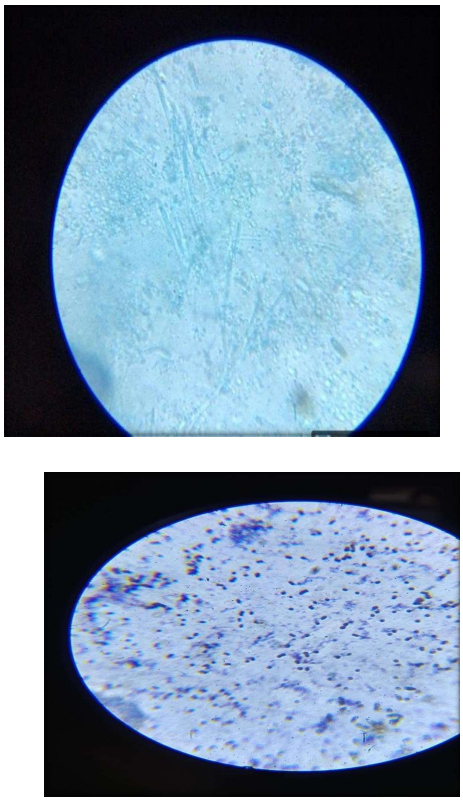
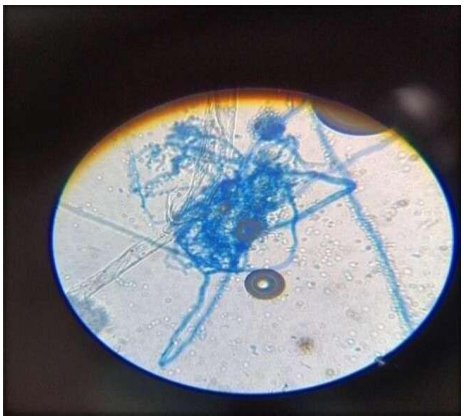


Figure 11. Representative isolates of *Micrococcus* and *Arthrobacter* recovered from *biological samples* following prolonged environmental exposure.

Soil and tile samples were harvested at the 24-hour exposure time, and fungal growth was detected in the form of a wide variety of *Aspergillus* species (fungal colonization at the later stages of environmental exposure).



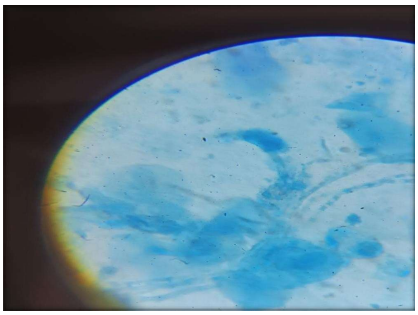


Figure 12. Representative isolates of *Aspergillus terreus* and *Nocardia* recovered from
biological samples maintained at 20–25°C.

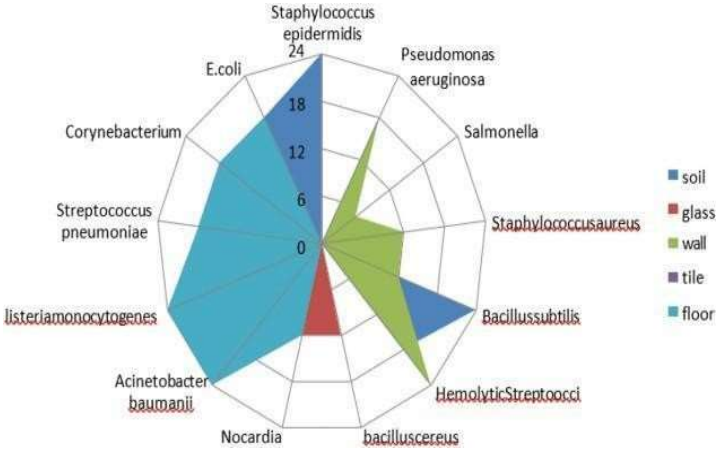


Figure 13. Distribution of *microorganisms* isolated from *blood samples* maintained at 20–25°C across different surfaces and exposure periods.

Saliva Samples

The pattern of microbial colonisation was similar in samples of saliva which were kept at ambient temperatures. Microbial diversity was found highest in soil, in general, across exposure. After 24 hours of exposure, there was no observable microbial growth that was detected on the surface of the wall. Under ambient conditions in the environment, however, the mixed population of bacteria and fungi was recovered from floor, tile and glass after 18 hrs, reflecting progressive microbial colonization under ambient environmental conditions.

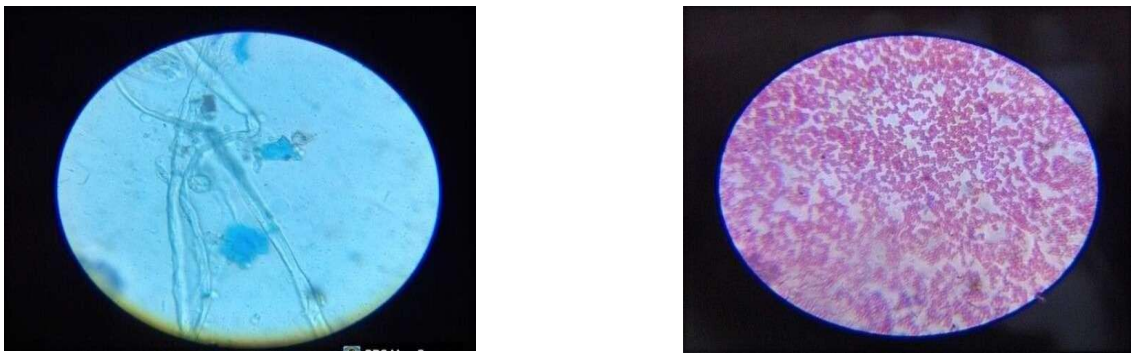


Figure 14. Representative isolates of *Candida* and *Pseudomonas aeruginosa* recovered from *saliva samples* maintained at 20–25°C.

Exposure for 24 hours resulted in a decrease in microbial recovery for all non-porous surfaces. This persistence of Acinetobacter under current environmental conditions is clear from being a dominant isolate on the glass surface.

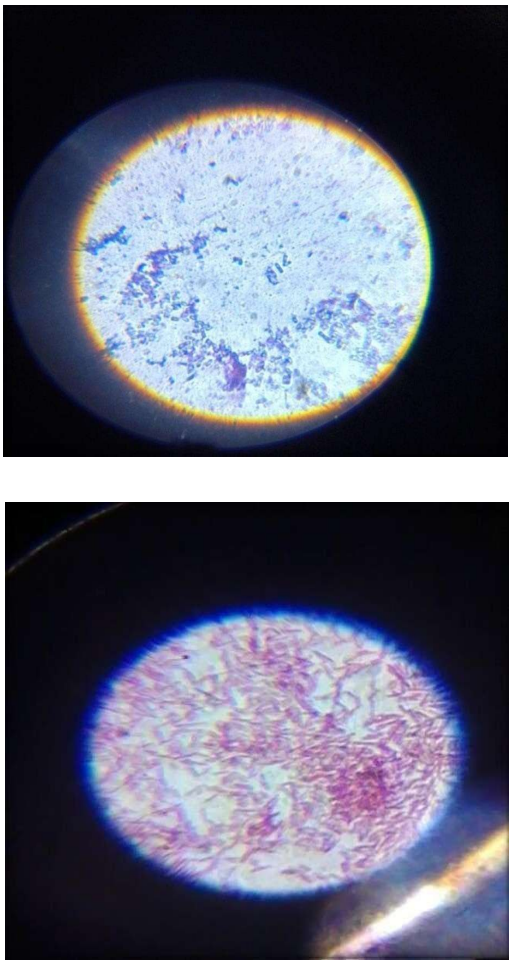


Figure 15. Representative isolates of *Staphylococcus epidermidis* and *Serratia marcescens* recovered from *saliva samples* following prolonged environmental exposure.

After 24 hours of environmental exposure, Nocardia spp. were isolated from the tile, wall and glass surfaces.

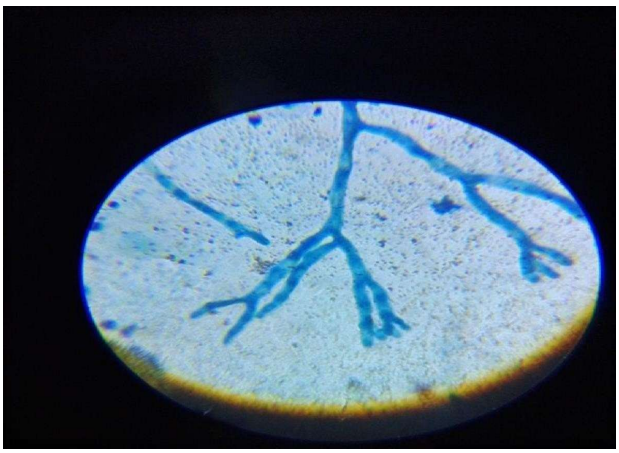


Figure 16. Representative isolate of *Nocardia* recovered from *saliva samples* following prolonged environmental exposure.

Blood samples from all experimental surfaces at 4–9 °C were mainly taken up with Gram-positive microorganisms. The microbial diversity of the soil showed no reduction during exposure and resulted in recovery of both Gram-positive bacteria and Gram-negative bacteria including Flavobacterium. In contrast, surfaces such as glass, wall, and ceramic tile had comparatively low colonization by microbes, and no bacterial isolates were recovered after 12 hours of exposure of these non-porous surfaces.

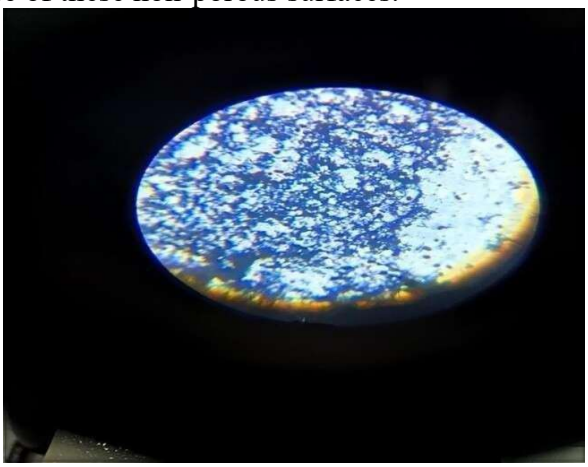


Figure 17. Representative isolate of *Flavobacterium* recovered from blood samples

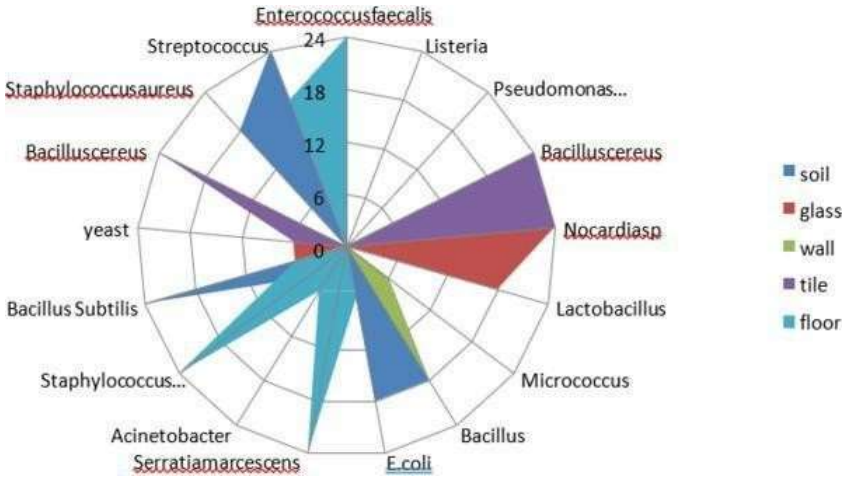


Figure 18. Distribution of *microorganisms* isolated from *saliva samples* maintained at 20–25°C across different surfaces and exposure periods.

Effect of Ambient Temperature (40–45°C) On Biological Fluids

Figure 19: *Culture Plates* isolated from *blood samples* maintained at 40–45°C across different surfaces
and exposure periods.

Blood Samples (40–45°C)

- After 48 hours mixed Gram-positive organisms as well as Gram-negative rods were seen.
- *Salmonella* and *Pseudomonas* were able to extend their lifespan to 48 hrs in soil.
- At 48 hours ,soil was dominated by *Aspergillus and Candida*.
- Gram-positive bacilli that form spores were present for as long as 48 hours in soil.
- Microbial growth on polymers and composites such as floor and tiles was found up to 24 hours while on glass and wall surfaces growth occurred up to 12 hours.
- Hemolytic *Streptococci* was cultured for a period of 24 hours in blood samples.

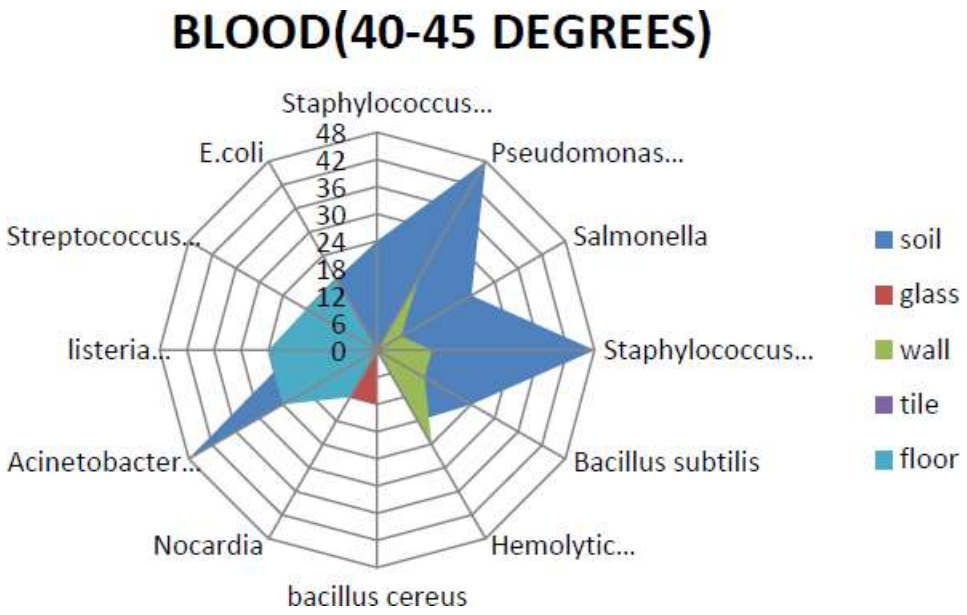


Figure 20: Distribution of *microorganisms* isolated from *blood samples* maintained at 40–45°C across different surfaces and exposure periods.

Saliva Samples (40-45°C)

- Soil was found to support *Lactobacillus, Enterococcus faecalis* and *Streptococcus* for up to 24 hours.
- *P. aeruginosa, Salmonella, S. aureus,* and *species of Bacillus* were detectable for 48 hours in soil.
- Soil was most rich in *Aspergillus* after four days.
- Microbial growth was observed for up to 12 hours on wall surfaces and 24 hours on tile, glass, and floor surfaces.

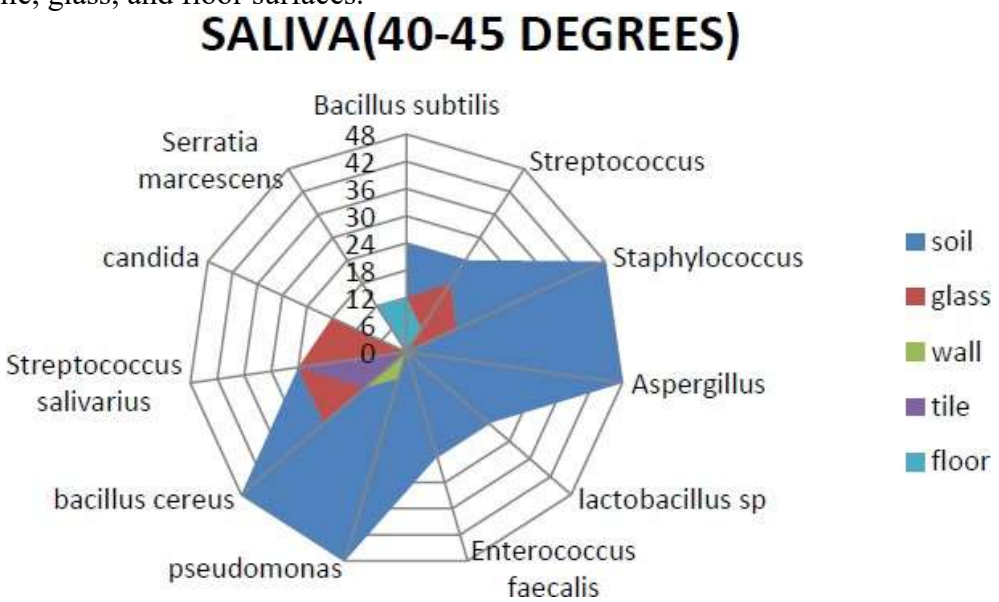


Figure 21: Distribution of *microorganisms* isolated from *saliva samples* maintained at 40–45°C across different surfaces and exposure periods.

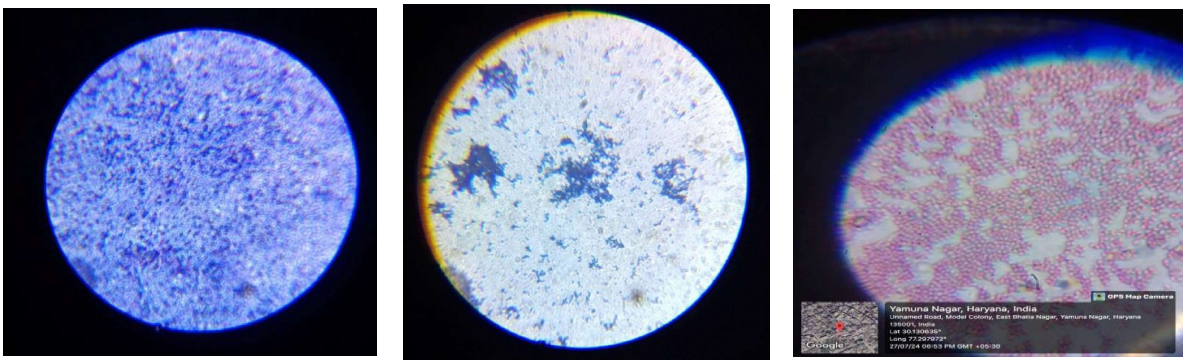


Figure 22. Representative photomicrographs of *Staphylococcus aureus, Bacillus cereus,* and
Enterococcus faecalis isolated from biological samples maintained at 40–45°C.

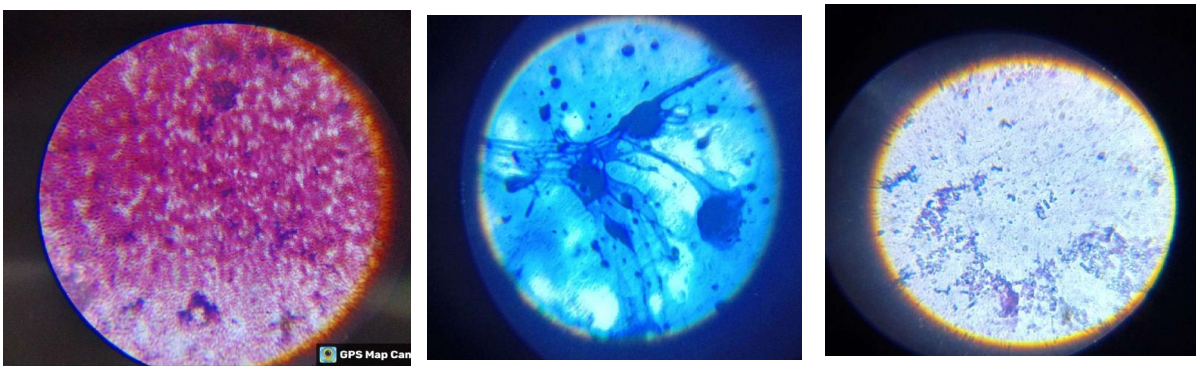


Figure 23. Representative photomicrographs of *Pseudomonas aeruginosa, Aspergillus* spp. And
Bacillus subtilis isolated from biological samples maintained at 40–45°C.

Overall Observations

Environmental temperature, exposure duration, and surface characteristics could affect microbial recovery. In all the calculated substrates, soil had the highest microbial diversity at the 4-9°C ,20-25 C and 40-45°C treatments. Microbial recovery was more favorable at lower temperatures (4–9°C) across most non-porous surfaces; Ambient temperatures (20–25°C) allowed for increased microbial proliferation and recovery, especially at the intermediate stages of exposure to the environment.

6. DISCUSSION

Within the Forensic examination, the preservation of biological evidence is crucial in performing a strong examination as biological fluid can start to undergo environmental alteration right after being deposited at a crime scene (Nasir et al., 2026). The results of the present research show that environmental temperature, surface properties and duration of exposure all influence microbial colonization of blood and saliva. These together will affect the biological degradation rate and ultimately the usefulness of evidence for further forensic examination.

Temperature was one of the main variables studied as it had a significant influence on the growth of microorganisms. Most of the samples held at ambient temperature (20-25°C) had significantly more growth of microorganisms than those kept at 4-9°C, with increasing numbers of bacteria and fungi found through the intermediate exposure periods. A significant increase in microbial growth was seen on most of the substrates exposed at ambient temperature (20-25°C) compared to those stored at 4-9°C, with bacteria and fungi present at increasing numbers throughout the intermediate exposure periods. The results of the latter showed, on the contrary, that lower temperatures considerably reduced microbial growth, thus helping to retard microbial degradation and preserve forensic evidence.

Surface type also affected the microbial colonization. The microbial diversity in the soil, both at low and high temperatures, was always the most diverse, which indicates that there are abundant microorganisms living in the soil that can quickly colonize biological fluids. Non-porous surfaces, such as glass, walls and ceramic tiles, however showed relatively little microbial persistence, especially when refrigerated conditions occurred. Based on this it is concluded that the preference of this property affects the survival of the microorganisms through affecting how the moisture behaved on the substrate, its nutrient availability and the stability of the microenvironment after deposition of the biological fluids.

Gracia-de-Lucas et al., (2026) noticed distinct blood and saliva degradation throughout environmental exposure. It was found that there was extensive microbial colonization and that hemolytic Streptococcus spp. were often found in advanced stages of degradation in the blood samples. Microbial contamination of the saliva was also shown to have been progressive, and after long periods, no amylase were detectable when left long in ambient conditions(Bagban et al., 2019). From these observations, it is clear that the initial chemical, physical and biological characteristics of biological evidence commonly studied at forensic facilities undergo gradual changes as a consequence of the metabolisms of microorganisms.

The length of time spent exposed was significantly linked to the biological degradation (Wahab et al., 2025). Microbes grew moderately over much of the substrates during the first exposure time. Microbacterial colonization increased with the duration of exposure with peaks at the intermediate exposure time. After that, microbial recovery was less on the non-porous surfaces presumably due to the progressive dryness that occurred on the biological stains.

From a forensics point of view, these results show the need for early evidence retrieval(Aloraer, 2017). When blood and saliva are collected late, it gives environmental microorganisms the time to invade the body and make the tests (serological, microbiological, molecular) unreliable. There is thus a need for continued control in the collection, preservation and storage of samples.

There are a number of limitations to the current study. The study has been limited to two ranges of the environment temperature and five prevalent surface types in regulated laboratory experimental conditions. Humidity, ultraviolet radiation, precipitation or seasonal changes in the climate was not studied and could have other effects on microbial colonization and degradation of biological fluids. Moreover, only blood and saliva were examined during the investigation, while other types of forensic bioevidence might have different degradation characteristics under similar environments.

Although there are some limitations in the study, it offers experimental evidence of the role of combined effects of temperature, surface substrate and exposure time on biological fluid degradation by microorganisms. These results contribute to the understanding of how the environment impacts forensic evidence, and offer guidance on evidence collection and

preservation. They also lay the groundwork for further research to build upon to create improved methods of biological degradation reduction and increase the certainty of forensic laboratory examinations.

7. CONCLUSION

The results of the present study show that the preservation of biological fluid of forensic relevance depends on the interaction between different environmental factors such as temperature, surface morphology and time of exposure. These results demonstrate that the microbial colonization and the degradation of the biological evidence are affected significantly by these environmental factors, in the experimental analysis applied to three illness-relevant deposit surfaces, five commonly encountered inanimate surfaces, under two temperatures. The study thus confirms the significance of knowledge of the environment in which a sample of a biological fluid has been found and that deterioration prior to the laboratory examination can significantly contribute to lowering the value and correcting power of the fluid samples found as a forensic object.

Of the variables tested, temperature proved to have the greatest impact on preserving biological fluids. Samples stored at ambient temperature (20–25°C) had a large increase in microorganisms during storage, causing enhanced digestion of both blood and saliva over the 10-day study. The pathogenic microorganisms were found to survive 40-45^C at 48 hours.In contrast, the temperatures typically used for refrigeration (4-9 C) were highly effective at limiting formation of unwanted bacteria, which showed that lower temperatures have an inhibitory impact on microbial metabolism and will slow down biological deterioration. The new findings emphasise the value and need to keep material at low temperatures when biological evidence has to be handled later than it can be processed for the laboratory for best preservation of sample integrity and successful forensic analytical results.

Surface characteristics also had an effect on the extent of microbial contamination. In both temperatures, the highest level of biological fluid degradation and the highest microbial diversity was maintained in soil. Favourable moisture holding and nutrient availability and the naturally high levels of microbes, allowed for quick microbial colonization. In contrast, most non-porous surfaces i.e. glass, painted walls, ceramic tiles and floors, showed lower microbial persistence, especially for the refrigerated conditions where the bacterial growth was significantly limited after the initial exposure periods. These observations reveal a direct link between the properties of the substrate and the survival of the microbes and highlight the importance of carefully considering substrate properties for planning for crime scene investigation, evidence recovery, and preservation.

The length of exposure also had an impact on the integrity of biological evidence. There was relatively little microbial growth in the early hours after deposition, meaning that blood and saliva may have a significant forensic value. Ruined biological fluids developed with the progressive colonization of microorganisms induced by prolonged exposure to environment, However, the temperature environment under which these experiments were conducted was ambient and the time interval from 12 to 24 hours showed the highest microbial growth rates, suggesting that the delays in evidence recovery increase the likelihood of degradation. Microbial recovery on a few non-porous surfaces throughout the later exposure period had reduced as a result of progressive drying, but high levels of microbial damage were detected prior to this level of reduction. This information has implications for the necessity to collect

evidence as soon as possible to avoid further degradation to the environment and the inaccuracy that such degradation could cause in later forensic examinations.

Microbial degradation patterns were also found; patterns in blood and patterns in saliva. The advanced state of blood degradation was associated with mentions of Hemolytic Streptococcus species, while a long time of microbial activity in saliva was correlated with a decrease in measurable amylase activity, an important biochemical substance that is frequently used in the everyday forensic analysis of body fluids. Change in these biological properties over time illustrates microbial metabolism can affect serological analyses, biochemical analyses, and molecular analyses, and makes biological samples collected from a crime scene less reliable.

From a practical forensic standpoint, the results present much useful information to forensic personnel involved in the recovery and preservation of biological evidence, forensic practitioners, and crime scene investigators. An understanding of temperature, substrate, and length of exposure in the degradation of microbes can help investigators prioritize evidence collection, preserve the specimen properly, and limit the contamination in transport and storage. Especially mention should be made of biological samples that have been placed on soil and other substrates on which extensive microbial growth can take place, where rapid deterioration is most likely to occur. It is, therefore, imperative that recovery is promptly made, appropriate packaging is adopted and environmental conditions can be controlled to preserve the integrity of the evidence and enhance reliability of the subsequent laboratory analysis.

However, the following limitations need to be recognised. The investigation was limited under controlled laboratory conditions to two biological fluids, two temperature ranges, and five types (or conditions) of surfaces encountered. The other environmental parameters such as humidity, ultra-violet radiation, rainfall, seasonal climatic changes and outdoor exposure,

were not included in the study but could also have an effect on the microbial succussion and biological fluid deterioration. In addition, conventional microbiological rather than molecular methods were used to identify microbes, rather than molecular techniques which could offer more taxonomic breakdown. Future research should thus consider a wider range of environmental scenarios, expanded and varied sample size, increased time and a wider variety of molecular identification techniques that may better convey how forensics evidence may degrade in real-world scenarios.

In general this study gives strong experimental evidence, suggesting the microbial degradation process of blood and saliva are affected synergically by the environmental temperature, the characteristics of the substrates and the duration of exposure. Results contribute to existing knowledge of the environmental factors that impact biological evidence preservation, and supply scientifically sound recommendations for the recovery, storage, and laboratory examination of evidence. It highlights the need for quick collection of evidence, proper preservation techniques and taking into consideration environmental conditions, which helps to improve the management of forensic evidence, reliable forensic laboratory analyses and ensure more accurate and defendable forensic investigations.

8. REFERENCES

Aloraer, D. (2017). Evaluation of collection protocols for the recovery of biological samples from crime scenes [University of Lancashire]. In *CLOK (University of Central Lancashire)*.

Bagban, M., Parmar, N., & Jain, N. (2019). EXTRACTION & PURIFICATION OF HEMOLYSIN FROM TOXIN PRODUCING MICROORGANISMS AND ITS EFFECT ON

FORENSICALLY IMPORTANT BLOOD AND SALIVA SAMPLES. *Bacterial Empire, 2*(4), 76–76.

Bisker, C., Taylor, G., Carney, H., & Ralebitso-Senior, T. K. (2021). A Combined Application of Molecular Microbial Ecology and Elemental Analyses Can Advance the Understanding of Decomposition Dynamics. *Liverpool John Moores University, 9*.

Divya, K., GK, M., Umema, A., & SS, D. (2024). Environmental Factors Affecting the Concentration of DNA in Blood and Saliva Stains: A Review. *Journal of Forensic Science and Research, 8*(1), 9–15.

Elliott, C., Shafer, A. B. A., & Stotesbury, T. (2022). The crux of time: A meta-analysis of ex vivo whole blood degradation. *Frontiers in Analytical Science, 2*.

Gracia-de-Lucas, S., López-Matayoshi, C., Lozano-García, M., & Gomes, C. (2026). Unearthing forensic genetics: preliminary results on the ability to generate DNA profiles from buried human biological samples. *International Journal of Legal Medicine*.

Iancu, L., Bonicelli, A., & Procopio, N. (2024). Decomposition in an extreme cold environment and associated microbiome—prediction model implications for the postmortem interval estimation. *Frontiers in Microbiology, 15*, 1392716–1392716.

Joseph, É. (2021). *Microorganisms in the Deterioration and Preservation of Cultural Heritage*.

Lim, D. V., Simpson, J. M., Kearns, E. A., & Kramer, M. F. (2005). Current and Developing Technologies for Monitoring Agents of Bioterrorism and Biowarfare. *Clinical Microbiology Reviews, 18*(4), 583–607.

Nasir, A. M., Kamal, N. H. N. M., & Hamzah, N. H. (2026). Forensic Reliability of Body Fluids in Sexual Assault Investigations: A Systematic Review. *Analytica—A Journal of Analytical Chemistry and Chemical Analysis, 7*(1), 21–21.

Ogdur, M., Çakan, H., & Çevik, F. E. (2018). Investigation of the microorganisms decaying bloody evidences. *Medicine Science | International Medical Journal, 1*–1.

Pajnič, I. Z. (2025). Analysis of Human Degraded DNA in Forensic Genetics. *Genes, 16*(11), 1375–1375.

Rankin-Turner, S., Turner, M. A., Kelly, P. F., King, R. S. P., & Reynolds, J. C. (2018). Transforming presumptive forensic testing: in situ identification and age estimation of human bodily fluids. *Chemical Science, 10*(4), 1064–1069.

Salzmann, A. P., Russo, G., Kreutzer, S., & Haas, C. (2021). Degradation of human mRNA transcripts over time as an indicator of the time since deposition (TsD) in biological crime scene traces. *Forensic Science International Genetics, 53*, 102524–102524.

Song, Y., Huang, Y.-Y., Liu, X., Zhang, X., Ferrari, M., & Qin, L. (2014). Point-of-care technologies for molecular diagnostics using a drop of blood. *Trends in Biotechnology, 32*(3), 132–139.

Swayambhu, M., Kümmerli, R., & Arora, N. (2023). Microbiome-Based Stain Analyses in Crime Scenes. *PubMed Central, 89*(1).

Upadhyay, M., Shrivastava, P., Verma, K., & Joshi, B. C. (2023). Recent advancements in identification and detection of saliva as forensic evidence: a review. *Egyptian Journal of Forensic Sciences, 13*(1).

Verdier, T., Coutand, M., Bertron, A., & Roques, C. (2014). A review of indoor microbial growth across building materials and sampling and analysis methods. *Building and Environment, 80*, 136–149.

Wahab, A. M. A., Menon, S., & Jacob, A. A. (2025). *Evaluation of Microbial Growth in Dried Blood Stains*. 3750–3753.

Zapata, F., Ossa, M. Á. F. de la, & García-Ruiz, C. (2014). Emerging spectrometric techniques for the forensic analysis of body fluids. *e_Buah, 64*, 53–63.