CINEFORUM
ISSN: 0009-7039
Vol. 66. No. S6, 2026 (Special Issue 6)

Degradation of Forensically Important Biological Fluids: A Comparative Study on Different Surfaces, Temperatures, and Time Intervals

Neetu Sharma[1] , Jaskaran Singh[2]

[1]Research Scholar, Geeta University, Naultha, Panipat, India
[2]Professor, Geeta University, Naultha, Panipat, India
Email: {msneetumehta, jaskaransingh630 } @gmail.com
Corresponding Author:
Jaskaran Singh
ORCID ID:- 0009-0000-1206-3904

Abstract
Biological fluids recovered from crime scenes are vulnerable to environmental and microbial degradation, which can reduce their forensic value before laboratory examination. Although previous studies have examined individual factors affecting biological evidence, limited experimental research has systematically investigated the combined influence of temperature, exposure duration, substrate characteristics, and microbial activity on the degradation of blood and saliva. This study addresses this gap by examining the microbial degradation of blood and saliva deposited on five commonly encountered substrates: soil, glass, wall, floor, and ceramic tile, under three temperature conditions (4–9°C, 20–25°C, and 40–45°C). Samples obtained from five healthy volunteers were examined at 6, 12, 18, 24, and 48 hours. Microbial growth and degradation were evaluated using conventional culture methods, staining techniques, selective media, and standard biochemical tests.
The findings indicated that temperature, exposure duration, and substrate characteristics substantially influenced microbial growth and biological-fluid degradation. Soil exhibited the greatest microbial diversity and degradation, whereas non-porous surfaces generally demonstrated comparatively limited microbial growth. Moderate temperature conditions (20–25°C) produced pronounced degradation, particularly between 12 and 24 hours. Reduced detectable amylase activity was associated with saliva degradation, while folate-dependent haemolytic Streptococcus species were identified among the major contributors to blood degradation. The study demonstrates that biological-fluid persistence is governed by the interaction of environmental and substrate-related factors. The findings address an important research gap and provide practical implications for the timely recovery, packaging, preservation, and forensic interpretation of biological evidence..

Keywords : Biological fluids, microbial degradation, blood, saliva, temperature, substrates, forensic evidence, evidence preservation, crime scene. .

INTRODUCTION

Biological evidence recovered from crime scenes plays a fundamental role in forensic investigation because it can assist in personal identification, reconstruction of criminal events, and establishing associations among victims, suspects, and crime scenes (Aloraer, 2017,Aloría, 2016). Among the various forms of biological evidence, blood and saliva are particularly important because they are frequently encountered in violent offences, assaults, burglaries, and other criminal incidents (Upadhyay et al., 2023). Their forensic utility, however, depends not only on their presence at the crime scene but also on their condition at the time of recovery and laboratory examination. Following deposition, biological fluids are continuously exposed to environmental influences that may alter their physical, cellular, biochemical, and microbial characteristics, thereby reducing their evidential value over time (Zapata et al., 2014).
Blood and saliva contain a range of cellular and biochemical constituents that are relevant to forensic examination. Blood contains plasma, blood cells, proteins, enzymes, and microorganisms, whereas saliva contains epithelial cells, proteins, enzymes, and microorganisms originating primarily from the oral cavity (Song et al., 2014; Upadhyay et al., 2023). These components may provide valuable information for DNA profiling, body-fluid identification, serological examination, and other forensic analyses. Once deposited at a crime scene, however, these biological constituents are no longer protected by their original biological environment and become susceptible to physical, chemical, and biological alterations. Consequently, the condition of a biological stain at the time of collection may differ substantially from its original state.
Among the environmental mechanisms responsible for deterioration, microbial activity represents an important factor. Environmental bacteria and fungi, together with microorganisms naturally associated with biological fluids, can utilize proteins, carbohydrates, lipids, and other organic constituents as sources of nutrients (Lim et al.,

2005; Ogdur et al., 2018). Microbial proliferation may progressively alter cellular structures, enzymatic activity, and biochemical composition and may ultimately contribute to nucleic-acid degradation. Such changes can affect presumptive and confirmatory testing, DNA profiling, and interpretation of biological evidence, particularly when samples remain exposed for extended periods before recovery and examination (Pjanič, 2025).

Temperature is a major environmental variable influencing microbial growth and decomposition. Microbial metabolism, survival, and multiplication are strongly dependent on surrounding temperature, with lower temperatures generally suppressing the growth of many microorganisms, while favourable moderate temperatures may enhance microbial proliferation and accelerate biological degradation (Bisker et al., 2021; Iancu et al., 2024). At elevated temperatures, additional physical and biochemical changes may occur that can further influence the stability of biological stains. Therefore, temperature should not be considered merely as a storage condition but as an important environmental determinant of the persistence and integrity of forensic biological evidence.

The characteristics of the surface on which biological material is deposited may further modify the degradation process. Porous and non-porous substrates differ in their capacity to retain moisture, organic material, and microorganisms (Nasir et al., 2026; Ogdur et al., 2018). Soil, for example, contains a diverse microbial community and may provide favourable conditions for rapid microbial colonization of biological fluids. In contrast, relatively smooth and non-porous materials such as glass, ceramic tiles, and painted walls may provide less favourable conditions for microbial persistence (Verdier et al., 2014). Differences in moisture retention, aeration, nutrient availability, and surface structure can therefore influence both microbial diversity and the rate at which biological evidence deteriorates.

Exposure duration represents another critical determinant of biological-fluid persistence. During the initial hours following deposition, biological fluids undergo natural drying and environmental adaptation. With increasing exposure time, microorganisms may colonize the deposited material and utilize its nutritional constituents, progressively modifying its physical and biochemical characteristics (Divya et al., 2024; Salzmann et al., 2021). Consequently, delays in evidence recovery may increase the likelihood of microbial alteration and compromise subsequent laboratory examination (Swayambhu et al., 2023). Understanding the relationship between exposure duration and microbial colonization is therefore essential for developing evidence-sensitive approaches to crime-scene recovery and preservation.

Despite growing interest in the degradation of forensic biological evidence, an important gap remains in the existing literature. Previous investigations have frequently concentrated on DNA degradation, individual environmental variables, or a single type of biological fluid under relatively controlled laboratory conditions (Divya et al., 2024; Elliott et al., 2022). Comparatively limited experimental research has examined blood and saliva simultaneously across different substrate types, exposure durations, and temperature conditions, particularly from the perspective of microbial colonization and degradation. The interaction among these environmental variables is especially relevant to forensic practice because biological evidence recovered from actual crime scenes may remain exposed to different surfaces and temperatures for varying periods before collection. Consequently, findings obtained from studies examining only one environmental factor may not adequately represent the complexity of degradation occurring under realistic crime-scene conditions.

The present study addresses this research gap by systematically investigating the microbial degradation of blood and saliva deposited on five commonly encountered inanimate substrates—soil, glass, wall, floor, and ceramic tile—under three temperature conditions (4–9°C, 20–25°C, and 40–45°C) and across exposure periods of 6, 12, 18, 24, and 48 hours. Microorganisms associated with the biological fluids were isolated and characterized using conventional culture methods, staining techniques, selective media, and standard biochemical tests. By examining the combined influence of temperature, substrate characteristics, and exposure duration, the study seeks to provide a more comprehensive understanding of the environmental factors governing microbial degradation of blood and saliva.

The study is theoretically grounded in an environmental degradation perspective, which proposes that the persistence of biological evidence is determined by the interaction between the biological characteristics of the deposited material and the surrounding environmental conditions. Within this perspective, temperature influences microbial metabolic activity, substrate properties determine microbial attachment and nutrient availability, and exposure duration provides the temporal opportunity for colonization and degradation. Accordingly, microbial degradation is conceptualized not as the consequence of a single factor but as a dynamic process resulting from the interaction of environmental conditions, substrate characteristics, microbial activity, and time. This integrated perspective provides a basis for understanding why the same biological fluid may demonstrate different degrees of deterioration when deposited on different surfaces or exposed to different temperature conditions.

By addressing the identified gap, the present study contributes empirical evidence concerning the comparative stability of blood and saliva under different environmental conditions. The findings may assist forensic practitioners in assessing the potential effects of environmental exposure on biological evidence and may support more appropriate decisions concerning the timing of recovery, collection, packaging, preservation, and laboratory examination of biological stains. Ultimately, a clearer understanding of microbial degradation patterns may

improve the interpretation and evidential reliability of biological material recovered from challenging crime-scene environments.

MATERIALS AND METHODS

Study Design

An experimental study was conducted to investigate the influence of environmental temperature, surface substrate, and exposure duration on the microbial degradation of biologically significant forensic fluids. The study specifically examined the persistence and microbial alteration of blood and saliva deposited on different flat, inanimate surfaces and others such as (Wall, Ceramic Tile, Floor, Glass and Soil) under controlled temperature conditions. The experimental design enabled comparison of microbial growth and degradation patterns across different biological fluids, substrates, temperatures, and exposure periods.

Biological Samples

Whole blood and saliva were selected because both are commonly encountered biological fluids in forensic investigations and may provide important evidence for subsequent laboratory examination (Falweisen et al., 2009; Sipsey et al., 2009). Blood and saliva samples were obtained from five healthy adult volunteers using standard aseptic procedures. Following collection, the specimens were immediately maintained under refrigerated conditions (<4°C) prior to experimental processing to minimize pre-analytical microbial contamination and maintain sample integrity before deposition onto the experimental substrates.

Reference Culture

To carry out biochemical characterization, reference type cultures were procured from "Microbial Type Culture Collection CSIR-IMTECH" 32-A,CHD.Gram negative microorganisms *Escherichia coli* (MTCC NO.40) and gram positive microorganisms *Bacillussubtilis* (MTCC NO.121)along with *Candidaalbicans* (MTCC NO.183) were taken. A series of standard laboratory assays was carried out to facilitate biochemical characterization with analysis and examination .

Ethical Approval

The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC), vide Letter No. IEC/ISFCP/2026/01/09. Only healthy adult volunteers who provided informed written consent were included in the study. Before participation, volunteers were informed about the purpose and procedures of the study, the nature of sample collection, potential risks and benefits, and their right to withdraw from participation at any stage without any adverse consequences. Participation was entirely voluntary.

All biological specimens were assigned unique identification codes to maintain participant confidentiality and privacy. No personally identifiable information was associated with the biological samples or microbiological observations used for the study. The samples and associated research information were used exclusively for research purposes and were handled in accordance with applicable ethical and confidentiality requirements.

Experimental Design

The experimental protocol was developed to investigate the combined influence of biological-fluid type, environmental temperature, substrate characteristics, and exposure duration on microbial colonization and degradation. Blood and saliva samples were separately deposited on five inanimate and other substrates commonly encountered in domestic and crime-scene environments: soil, glass, wall, floor, and ceramic tile.

Three controlled temperature ranges were used:

Low-temperature range: 4–9°C

Ambient-temperature range: 20–25°C

High-temperature range: 40–45°C

Following deposition, the biological fluids were exposed to the experimental conditions for five predetermined periods: 6, 12, 18, 24, and 48 hours. Thus, the experimental design incorporated two biological-fluid types, three temperature ranges, five substrate types, and five exposure periods. Based on the experimental conditions described, a total of 150 experimental samples were included in the study.

This factorial arrangement permitted comparative assessment of microbial degradation patterns across different combinations of temperature, substrate, biological-fluid type, and exposure duration.

Sample Collection Following Environmental Exposure

Following deposition, the biological samples remained undisturbed on the respective substrates for the predetermined exposure periods. Samples were collected aseptically at 6, 12, 18, 24, and 48 hours using sterile cotton swab applicators. A separate sterile swab was used for each sampling event to minimize the possibility of cross-contamination between samples and experimental conditions.

Immediately following collection, the swabbed specimens were transferred for microbiological examination. The same sampling procedure was consistently applied across all experimental conditions, including biological-fluid type, substrate, temperature range, and exposure duration. Standardization of the collection procedure was intended to ensure comparability of microbial observations across the experimental groups and to minimize variation attributable to differences in sample recovery.

Table1:SummaryofSampling:

SAMPLE (Biological fluid)	TEMPERATURE	SURFACE	TIME INTERVAL
Blood & Saliva	4-9 ^{0}C 20-25^{0}C 40-45 ^{0}C	Soil	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
Blood & Saliva	4-9^{0}C 20-25^{0}C 40-45 ^{0}C	Glass	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
Blood & Saliva	4-9^{0}C 20-25^{0}C 40-45 ^{0}C	Wall	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
Blood & Saliva	4-9^{0}C 20-25^{0}C 40-45 ^{0}C	Floor	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours
Blood & Saliva	4-9^{0}C 20-25^{0}C 40-45^{0}C	Ceramic Tile	6 Hours 12 Hours 18 Hours 24 Hours 48 Hours

Isolation of Microorganisms

The recovered biological samples were subjected to microbiological culture for the isolation and subsequent characterization of bacterial and fungal microorganisms. Nutrient agar was used for bacterial isolation, whereas Sabouraud's dextrose agar (SDA) was used for fungal isolation. All inoculated culture media were incubated under aerobic conditions in accordance with standard microbiological laboratory procedures.

For soil samples, microbial isolation was performed using the serial dilution technique. Soil suspensions were prepared using sterile 0.85% sodium chloride solution and serially diluted from (10^1) to (10^7). Aliquots of 10µL from the respective dilutions were inoculated onto Nutrient agar and Sabouraud's dextrose agar using the spread-plate technique. Following incubation, representative bacterial and fungal colonies displaying distinct morphological characteristics were selected and subcultured for further identification and characterization.

Identification of Microorganisms

Microbial isolates were identified using a combination of conventional staining, microscopic examination, motility testing, and standard biochemical characterization procedures. Bacterial isolates were subjected to Gram staining to determine their Gram reaction and cellular morphology, whereas fungal isolates were examined using Lactophenol Cotton Blue (LPCB) staining for assessment of their microscopic characteristics. Motility testing was additionally performed for selected bacterial isolates where required for identification.

Biochemical characterization of bacterial isolates was conducted using a panel of conventional laboratory tests, including:

Indole test
Methyl Red (MR) test
Voges–Proskauer (VP) test
Citrate utilization test
Urease test
Nitrate reduction test
Catalase test
Coagulase test
Triple Sugar Iron (TSI) agar test
Oxidase test
Where further differentiation was required, selected isolates were cultured on appropriate selective and differential media, including Blood agar, Chocolate agar, MRS agar, Mannitol Salt agar, Starch agar, MacConkey agar. The resulting cultural, microscopic, and biochemical characteristics were considered collectively for the identification and characterization of the recovered microorganisms.

Assessment of Biological Fluid Degradation

Microbial colonization and associated degradation were assessed across all experimental combinations of biological-fluid type, substrate, temperature, and exposure duration. The microbial growth observed following each predetermined exposure period was recorded and compared across the experimental conditions.

Changes in microbial activity over time were examined to characterize the progression of biological-fluid degradation. Comparative assessment of the results enabled evaluation of the influence of temperature, substrate characteristics, and exposure duration on microbial proliferation and the preservation of blood and saliva. The observed degradation patterns were subsequently considered in relation to the potential implications for the recovery, preservation, and forensic examination of biological evidence.

RESULTS

Microbial Degradation Under Low-Temperature Conditions (4–9°C)

The microbial recovery and degradation patterns of biological fluids were assessed under low-temperature conditions (4–9°C) across the different experimental substrates and exposure periods. The results demonstrated variation in microbial recovery according to the type of substrate and duration of exposure.

Blood Samples

Blood samples recovered from the experimental substrates at 4–9°C were predominantly associated with Gram-positive microorganisms. The greatest microbial diversity was observed on soil throughout the exposure period, where both Gram-positive and Gram-negative bacterial isolates were recovered.

In comparison, microbial recovery from the non-porous substrates was considerably lower. On glass, wall, and ceramic tile surfaces, bacterial recovery decreased with increasing exposure duration, with no detectable bacterial growth observed after 12 hours under the experimental conditions. On the glass surface, Gram-positive spore-forming bacilli and Gram-positive cocci were recovered at the 6-hour exposure period, whereas recovery became limited at subsequent sampling intervals.

On the floor surface, Gram-positive cocci were recovered up to 12 hours of exposure. Following this period, no further bacterial recovery was observed from the floor samples. Overall, the findings under low-temperature conditions indicated that soil supported greater bacterial diversity and longer microbial persistence than the comparatively non-porous surfaces examined in the study.

The distribution of microorganisms recovered from blood samples maintained at 4–9°C across the different substrates and exposure periods is presented in Figure 1, 2.

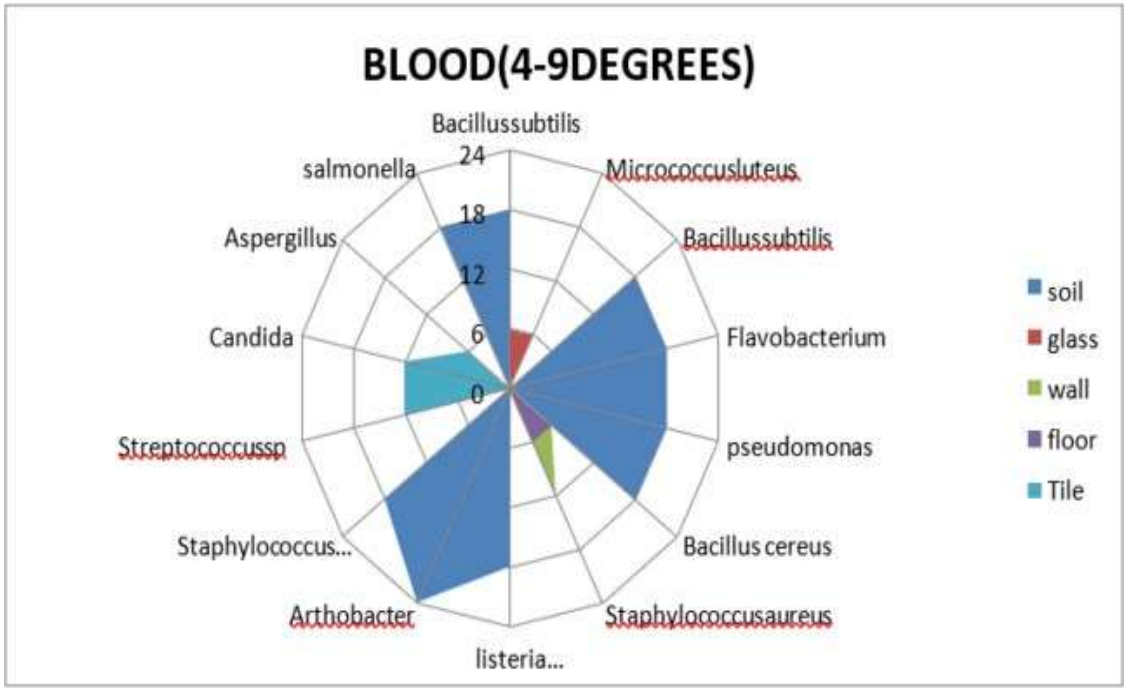
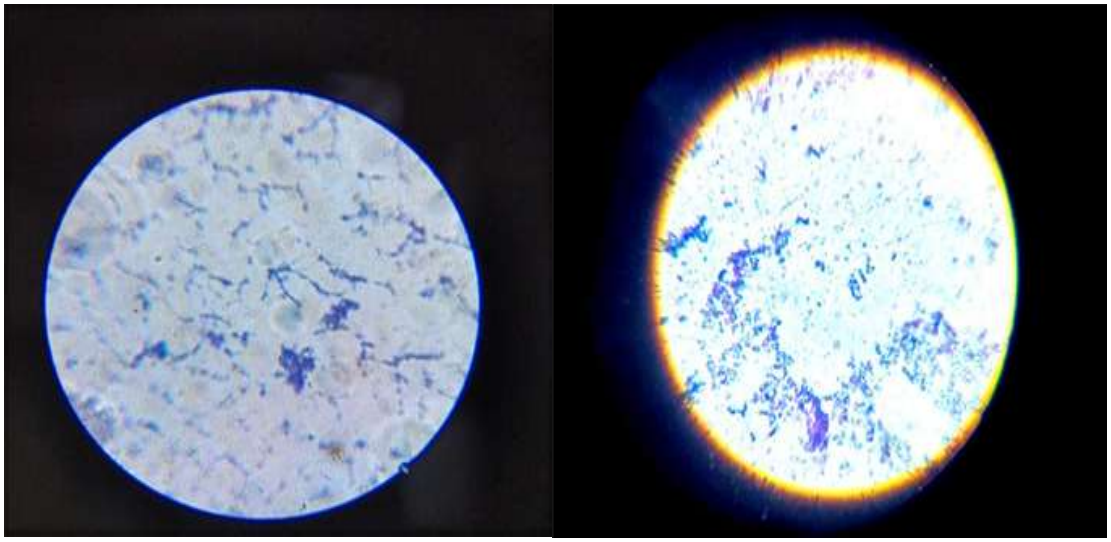
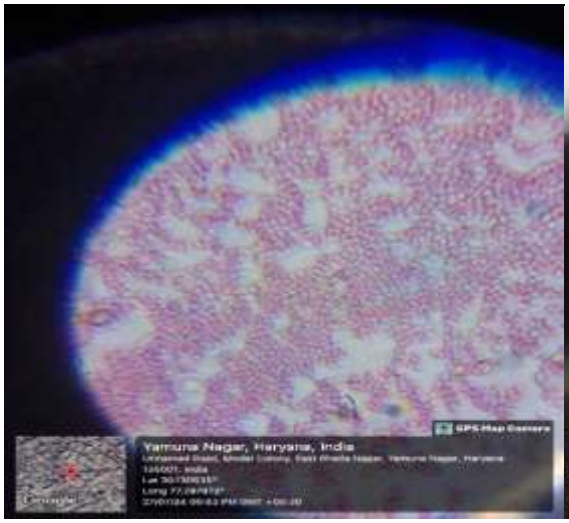


Figure 1. Distribution of microorganisms isolated from blood samples maintained at 4–9°C across different surfaces and exposure periods.

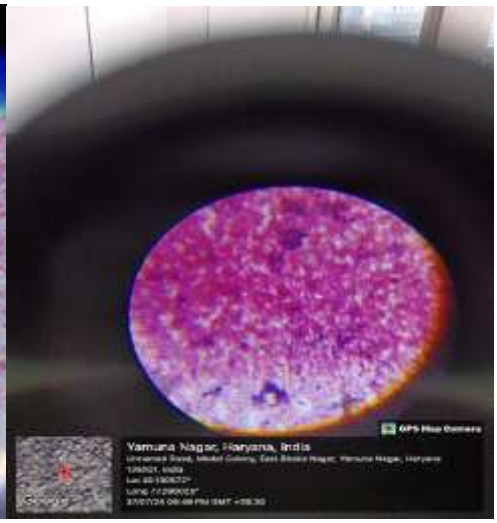
Mainly Gram positive microorganisms *Bacillus subtilis,Micrococcus,Streptococcus,Staphylococcus,Bacillus cereus* predominated in soil along with Gram negative *Pseudomonas,Salmonella,Flavobacterium* in soil. No bacterial growth was seen after 12 hours from glass, wall and tile surface. *Arthrobacter* was seen in soil samples upto 24 hours. Fungal growth of *Candida* and *Aspergillus* upto 12 hours observed.



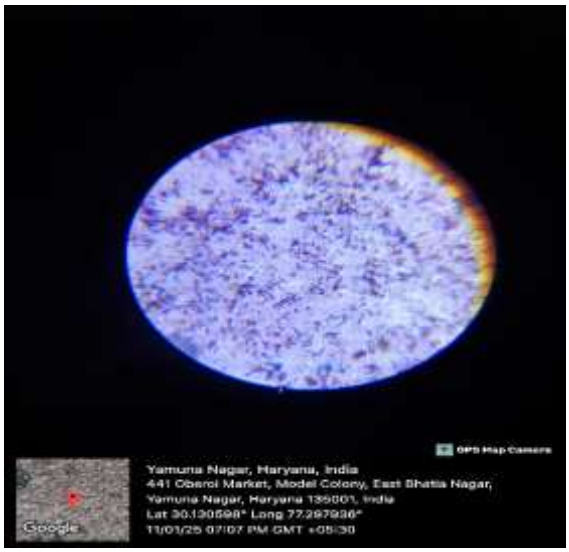
(b)



(c)

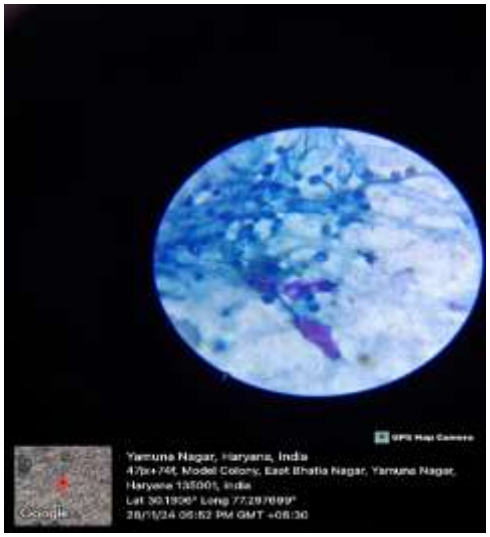


(d)



(e)

(f)



(g)

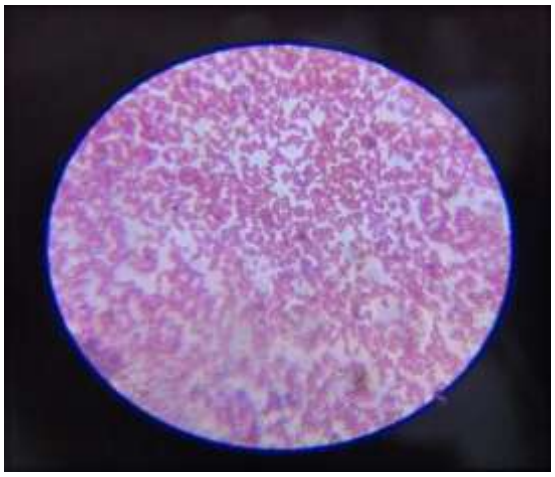
Figure 2. Representative isolates of *Bacillus cereus(a)* on floor/soil/wall, *Bacillus subtilis (b)*from soil/glass/ceramic,tile Enterococcus fecalis *(c)* from soil, *Pseudomonas (d)* from soil, *Staphylococcus sp (e)* from soil/floor/wall, *Streptococci (f) from ceramic tile ,Candida (g)* from soil/ceramic tile recovered from blood samples on soil surface maintained at 4–9°C.

Saliva Samples

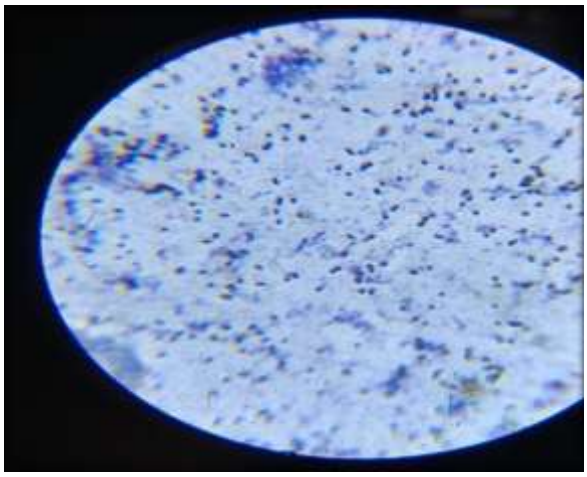
Microbial recovery from saliva samples under low-temperature conditions (4–9°C) was comparatively limited across the experimental substrates. Fungal isolates were detected on the ceramic tile surface during the early exposure period. *Aspergillus* species were recovered after 6 hours of exposure, whereas *Candida* species were detected after 12 hours. These findings indicate that, although overall microbial growth was limited under refrigerated conditions, selected fungal organisms remained recoverable during the exposure period.

The observed fungal recovery under low-temperature conditions demonstrated variation according to both exposure duration and substrate. In particular, the persistence of fungal isolates on the ceramic tile surface indicates that microbial survival may occur even under relatively low-temperature environmental conditions.

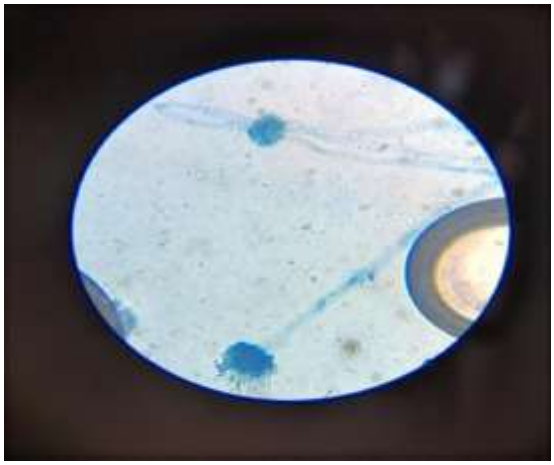
On the glass surface, *Bacillus cereus* was recovered at the 6-hour exposure interval, whereas *Bacillus subtilis* was recovered at 12 hours. This temporal difference in recovery indicates a change in the bacterial species detected on the substrate during environmental exposure. The observation suggests that the microbial profile associated with the deposited blood sample may vary with increasing exposure duration under low-temperature conditions.



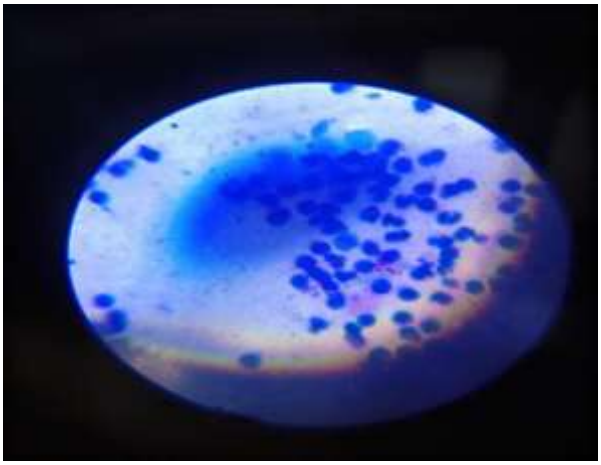
(h)



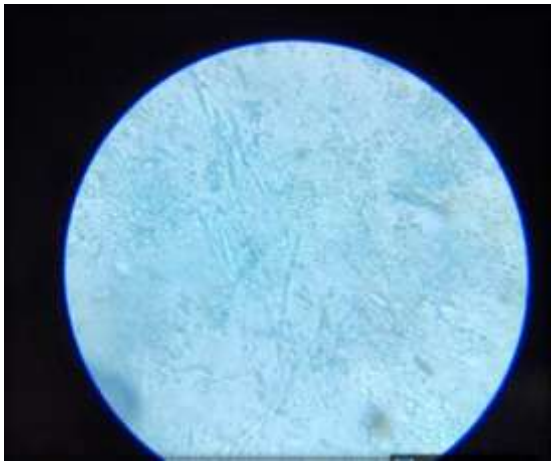
(i)



(j)



(k)

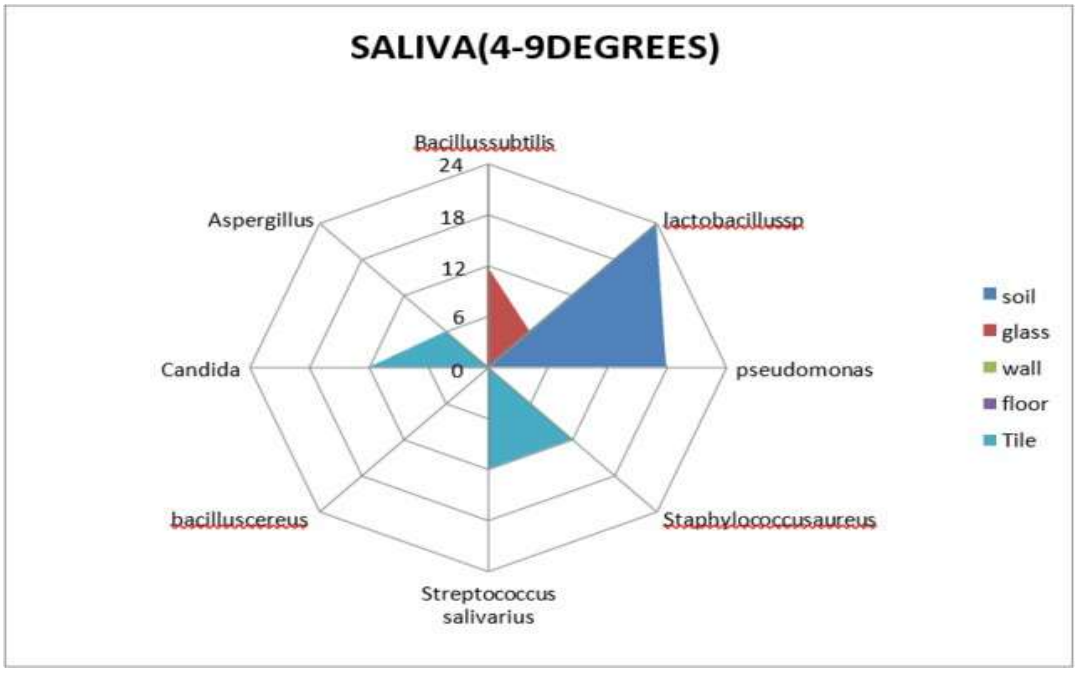


(l)

Figure 3. Representative isolates of *Pseudomonas aeruginosa(h) from soil/tile surface* ,*Lactobacillus* spp.(i) from soil/Ceramic tile,Aspergillus niger (j) from soil,Candida albicans (k) from soil/glass, *Streptococcus salivarius*(l) from soil/ceramic tile recovered from saliva samples maintained at 4–9°C

In soil samples, *Pseudomonas* spp. and *Lactobacillus* spp. remained detectable for comparatively longer exposure periods under the low-temperature condition (4–9°C).
Pseudomonas spp. were recovered up to 18 hours, whereas *Lactobacillus* spp. remained detectable up to 24 hours of environmental exposure. These observations indicate greater persistence of these microorganisms in soil compared with the shorter recovery periods observed on several of the other substrates under the same temperature conditions.

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



Growth of *Lactobacillus* was found upto 24 hours and *Pseudomonas* upto 18 hours in soil samples with saliva laden on it. *Bacillus subtilis*, a spore former,was able to survive at glass upto 12 hours followed by growth of *Streptococcus salivarius ,Staphylococcus aureus and Candida* on tile/soil surface.

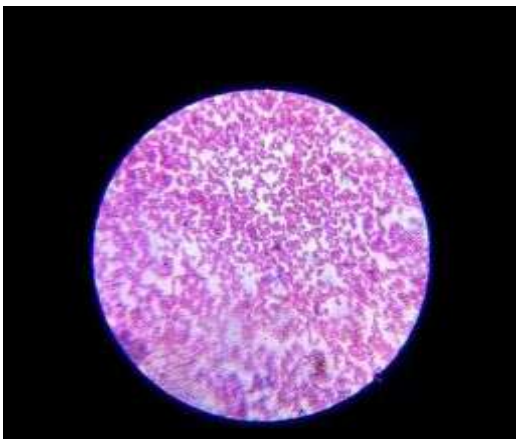
Effect of Ambient Temperature (20–25°C) On Biological Fluids

The microbial degradation of blood and saliva under ambient-temperature conditions (20–25°C) was assessed across the five experimental substrates and five exposure periods. Overall, microbial recovery increased with increasing exposure duration, although the magnitude and persistence of microbial growth varied according to the substrate.

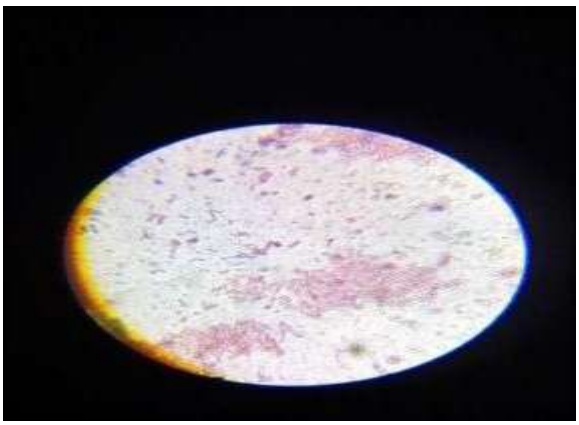
Figure 5.Culture Platesisolated from blood samples maintained at 20–25° Cacross different surfaces and exposure periods.

Blood Samples

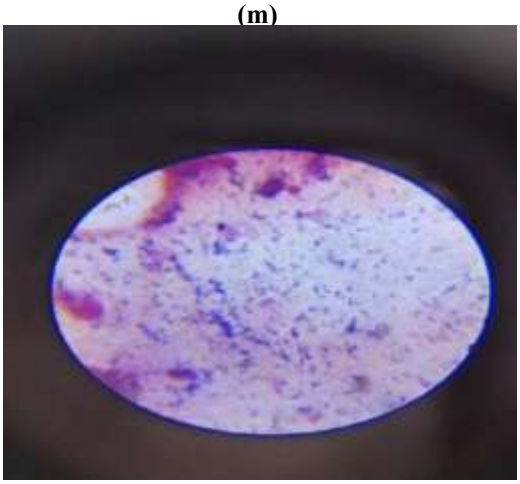
At 20–25°C, microbial recovery from the blood samples increased with increasing exposure duration. A greater level of microbial growth was observed at 24 hours compared with 12 hours across most of the experimental substrates. Throughout the observation period, soil demonstrated the greatest microbial diversity and the highest level of microbial recovery among the substrates examined (Figure 6).



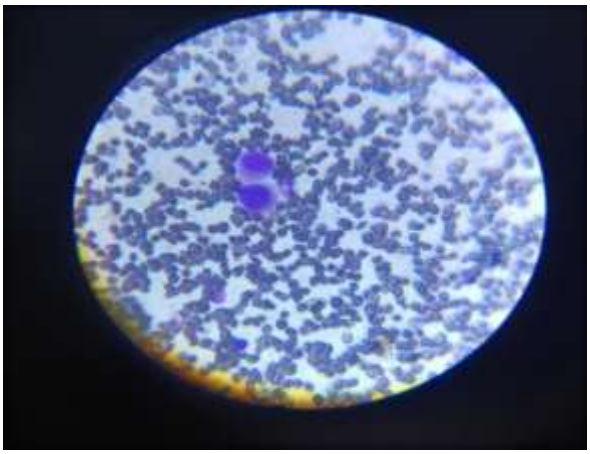
(m)



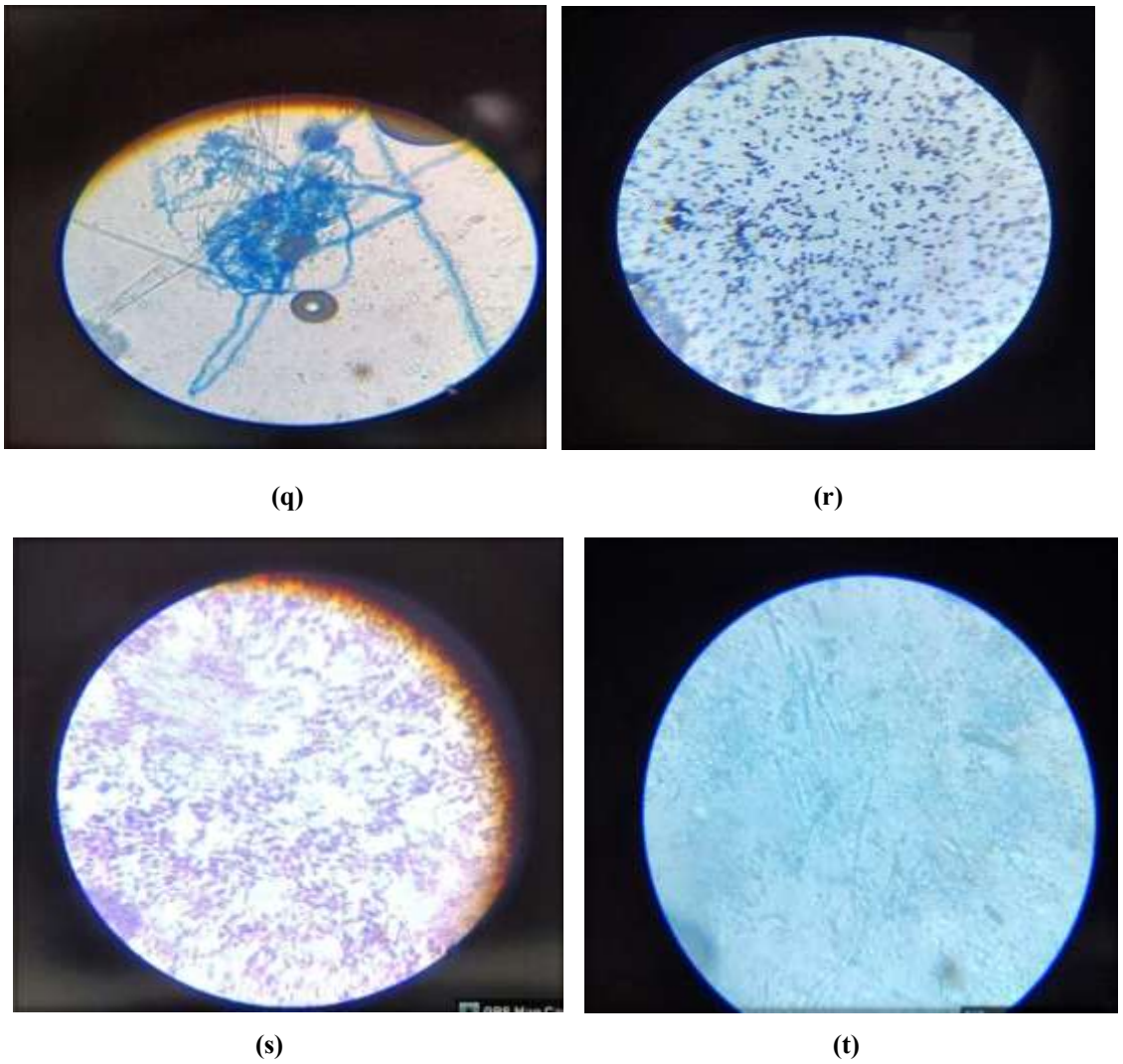
(n)



(o)



(p)



(q) **(r)**

(s) **(t)**

Figure 6. Representative isolates of *Enterobacter(m)from soil Pseudomonas(n),E.coli (o)from soil/floor/wall, ,Staphylococcus aureus(p) from soil/floor/wall, Aspergillus terreus (q) from soil/glass,Streptococcus pneumonia(r) from soil/glass,Streptococci hemolytic (s) from soil/floor, and Nocardia (t) from soil/glass on blood samples kept at 20-25^{0}C.*

A temporal variation in the bacterial profile was observed during environmental exposure. During the earlier exposure periods, Gram-negative cocci and bacilli were recovered, whereas the later exposure periods showed an increased representation of Gram-positive rods and cocci. This change indicated variation in the bacterial populations recovered from the blood samples as exposure duration increased.

No detectable microbial growth was observed on the wall surface after 24 hours of exposure under the experimental conditions. Among the microorganisms recovered from blood samples, haemolytic *Streptococcus* spp. were frequently isolated and were observed particularly during the later stages of exposure.

After 18 hours of exposure, samples recovered from the floor, ceramic tile, and glass surfaces showed mixed bacterial and fungal recovery. The presence of multiple microbial groups at this exposure period indicated continued microbial recovery from these substrates under ambient-temperature conditions.

At the 24-hour exposure interval, microbial recovery from the glass, wall, floor, and ceramic tile surfaces showed an overall reduction compared with earlier exposure periods. However, *Bacillus cereus* remained a prominent isolate on the glass surface despite the overall decrease in microbial recovery.

At 24 hours, samples recovered from the soil and ceramic tile surfaces demonstrated fungal growth. A range of *Aspergillus* spp. was observed among the fungal isolates, indicating fungal recovery during the later stages of environmental exposure.

The overall distribution of microorganisms recovered from blood samples maintained at 20–25°C across the different substrates and exposure periods is presented in Figure 13.

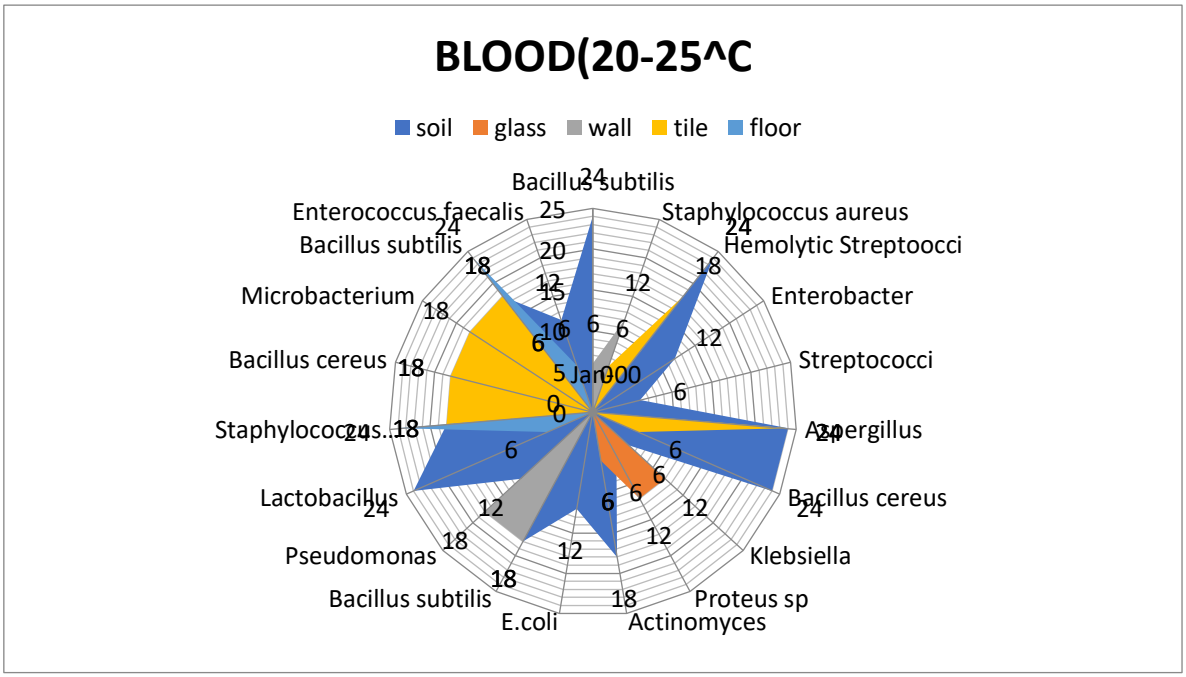


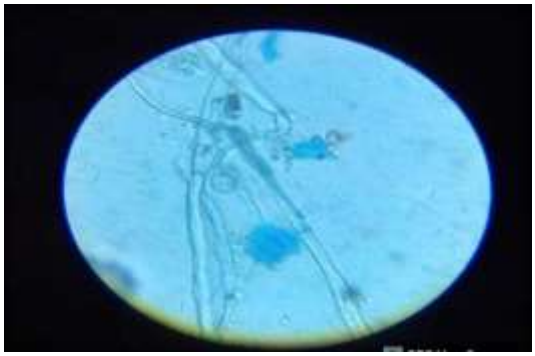
Figure7. Distribution of *microorganisms* isolated from *blood samples* maintained at 20–25° across different surfaces and exposure periods.

Growth of *Staphylococcus aureus,Bacillus subtilis,Aspergillus Bacillus cereus,Lactobacillus* were found upto 24 hours in soil samples**.***Staphylococcus aureus* was found on all surfaces except glass while *Bacillus subtilis* inhabited all surfaces.*Staphylococcus epidermidis* was found to survive on soil, ceramic tile and floor. *Enterococcus faecalis* was found on soil and floor surfaces. Glass surface supported growth of *Klebsiella,Proteus,Bacillus subtilis and Actinomyces.Hemolytic Streptococci* was predominant on soil, tile and floor.

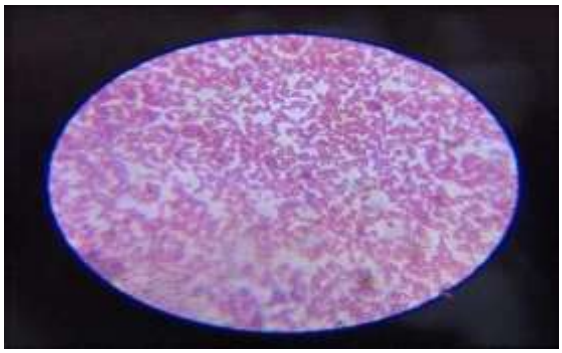
Saliva Samples

The microbial recovery pattern observed in saliva samples under ambient-temperature conditions (20–25°C) showed variation according to substrate and exposure duration. Among the substrates examined, soil demonstrated the greatest microbial diversity throughout the observation period. In contrast, no detectable microbial growth was observed on the wall surface after 24 hours of exposure under the experimental conditions.

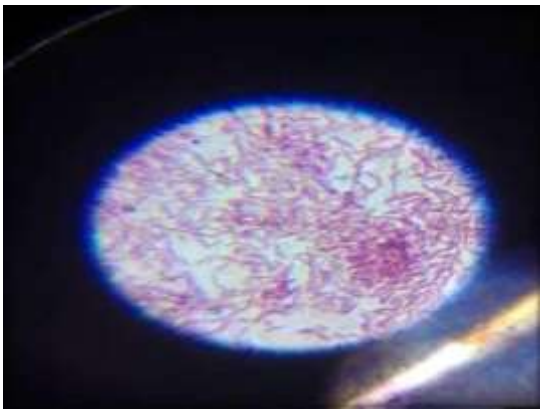
After 18 hours of environmental exposure, mixed populations of bacterial and fungal microorganisms were recovered from the floor, ceramic tile, and glass surfaces. The recovery of multiple microbial groups from these substrates indicated continued microbial presence during the exposure period under ambient-temperature conditions.



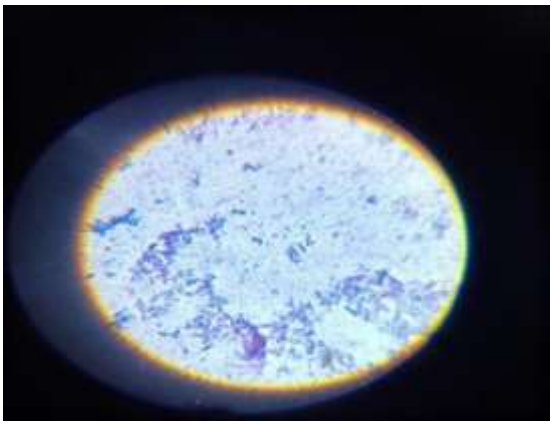
(u)



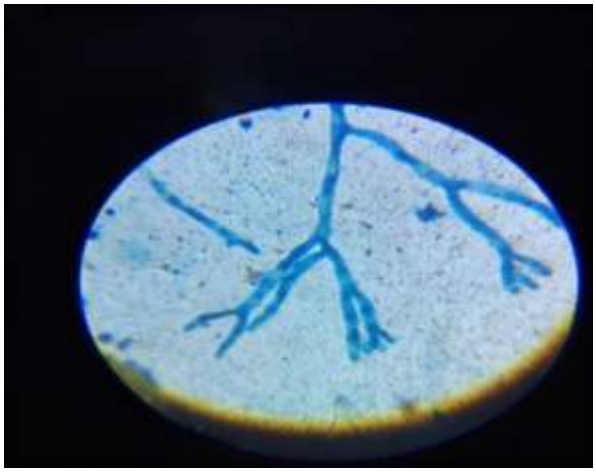
(v)



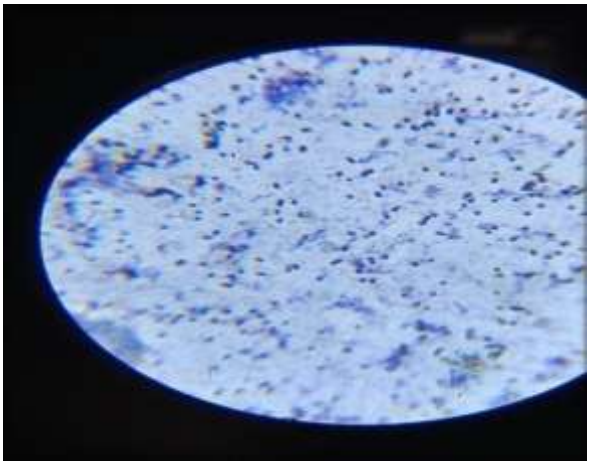
(w)



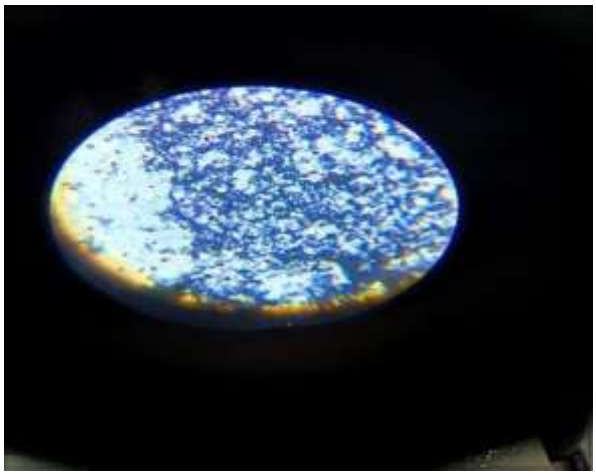
(x)



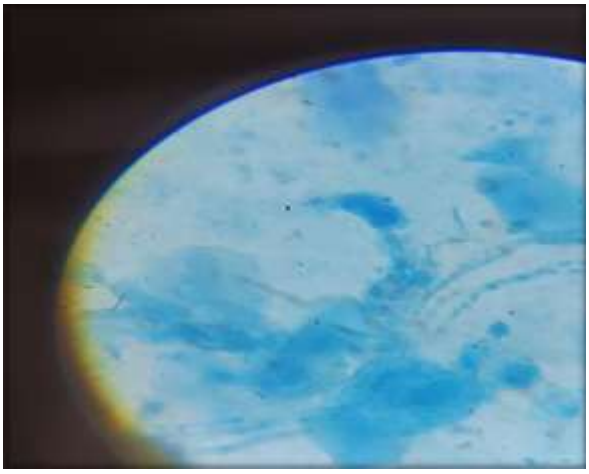
(y)



(z)



(a1)



(a2)

Figure 8. Representative isolates of Candida (u) from soil/floor , *Pseudomonas* (v) from soil/floor ,*Staphylococcus epidermidis* (x) ,*Serratia marcescens* ((w) from soil/floor,*Nocardia* (y) from Soil/ceramic tile/glass, *Micrococcus* (z) and *Arthrobacter* (a2)from soil/wall, *Flavobacterium* (a1) from soil surface at 20-25 °C.

At the 24-hour exposure interval, microbial recovery from the non-porous surfaces generally decreased compared with earlier exposure periods. Despite this overall reduction, *Acinetobacter* spp. remained detectable on the glass surface and represented one of the prominent bacterial isolates recovered from this substrate.
Following 24 hours of environmental exposure, *Nocardia* spp. were recovered from the tile, wall, and glass surfaces. Their recovery at the later exposure interval demonstrated the continued presence of selected microorganisms on these substrates despite the overall reduction in microbial recovery observed at 24 hours.
Under low-temperature conditions (4–9°C), blood samples recovered from the experimental substrates were predominantly associated with Gram-positive microorganisms. Soil demonstrated the greatest microbial diversity throughout the exposure period, with recovery of both Gram-positive and Gram-negative bacteria, including *Flavobacterium* spp. In contrast, comparatively limited microbial recovery was observed from the glass, wall, and ceramic tile surfaces. No bacterial isolates were recovered from these non-porous substrates after 12 hours of exposure under the experimental conditions.
The recovery of *Flavobacterium* spp. from soil further demonstrated the presence of Gram-negative bacterial isolates alongside the predominantly Gram-positive microbial population observed under the low-temperature condition.
The overall distribution of microorganisms recovered from saliva samples maintained at 20–25°C across the different substrates and exposure periods is presented in Figure 18.

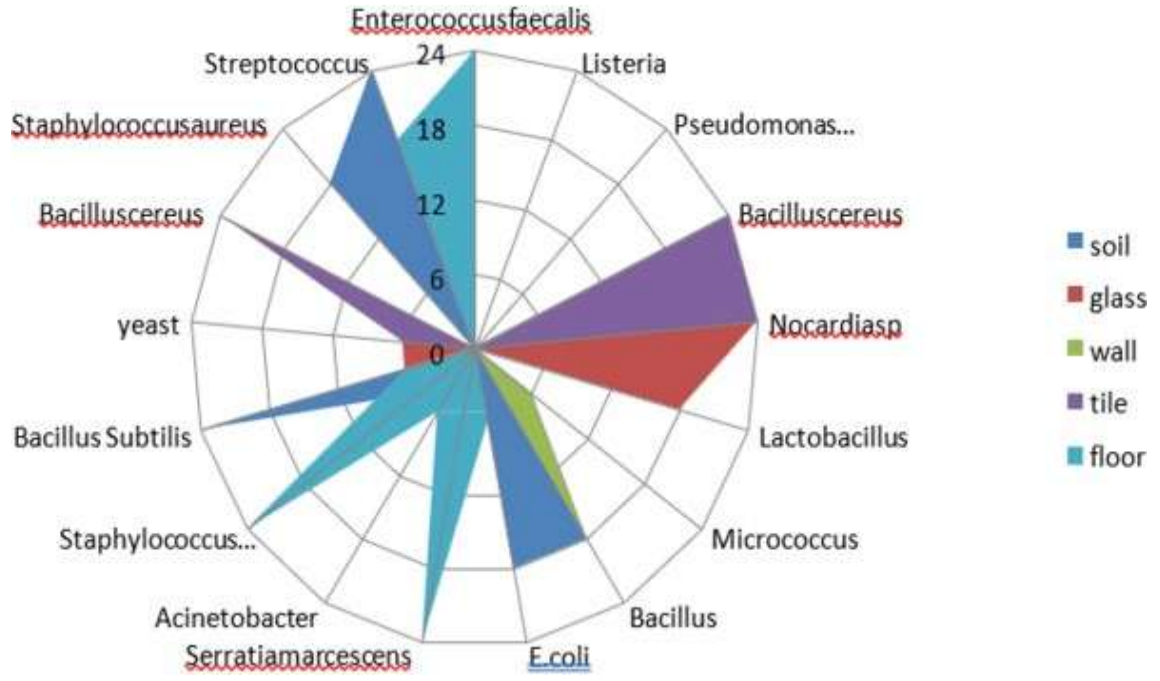


Figure9. Distribution of *microorganisms* isolated from *saliva samples* maintained at 20–25°C across different surfaces and exposure periods.

Streptococcus,E.coli,Bacillus sp were found predominantly in soil while *Nocardia* and *Staphylococcus epidermidis* were found on every surface. *Acinetobacter* was found to survive on glass surface while Yeast was found on tile and glass surfaces both.*Listeria* and *Serratia* was encountered on tile and floor surfaces at 20-25 ^{0}C.
Effect of HigherTemperature (40–45°C) On Biological Fluids
The microbial recovery and degradation patterns of blood and saliva were assessed under high-temperature conditions (40–45°C) across the experimental substrates and exposure periods. The results demonstrated variation in microbial persistence according to substrate type and duration of environmental exposure.

Figure 10:*CulturePlates*isolatedfrom*bloodsamples*maintainedat40–45°Cacrossdifferent surfaces and exposure periods.

Blood Samples (40–45°C)

Under the 40–45°C experimental condition, microbial recovery from blood samples varied according to substrate and exposure duration. At the 48-hour exposure interval, both Gram-positive microorganisms and Gram-negative bacilli were recovered from the experimental samples.

In soil, *Salmonella* spp. and *Pseudomonas* spp. remained detectable up to 48 hours of exposure. At the 48-hour interval, fungal isolates including *Aspergillus* spp. and *Candida* spp. were also recovered from soil samples. Gram-positive spore-forming bacilli remained detectable in soil throughout the 48-hour exposure period.

Microbial recovery from the floor and ceramic tile surfaces was observed for up to 24 hours, whereas recovery from glass and wall surfaces was limited to 12 hours under the experimental conditions. These findings demonstrated variation in microbial persistence across different substrates at high temperature.

Haemolytic *Streptococcus* spp. were recovered from blood samples for up to 24 hours of exposure. Overall, the microbial profile under the 40–45°C condition showed variation in both bacterial and fungal recovery according to substrate and exposure duration.

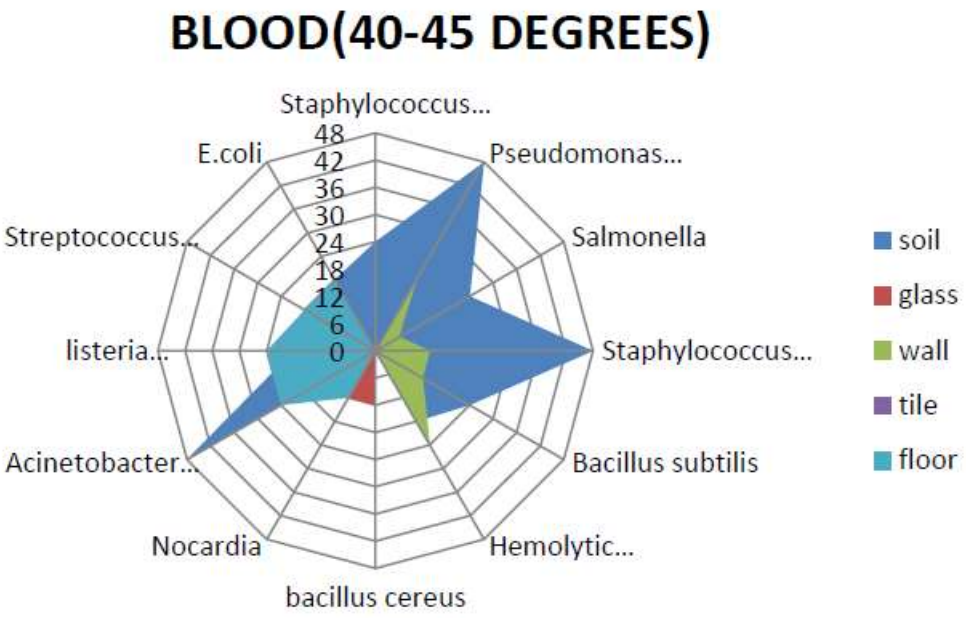


Figure 11 :Distribution of *microorganisms* isolated from *blood samples* maintained at 40–45°C across different surfaces and exposure periods.

Salmonella and *Pseudomonas* sp.along with spore former,*Bacillus subtilis* and fungi,*Aspergillus niger* and *Candida* survived 40-45 ^{0}C and last upto 48 hours on blood samples spread on soil and floor surfaces. Hemolytic Streptococci was found to inhabit wall surfaces along with *Pseudomonas and Staphylococcus aureus*.Glass surface contained population of *Nocardia* and *Bacillus cereus* at high temperatures.

Saliva Samples (40-45°C)
Under the high-temperature condition (40–45°C), microbial recovery from saliva samples varied across the experimental substrates and exposure periods. Soil demonstrated the greatest persistence and diversity of microorganisms among the substrates examined.
In soil samples, *Lactobacillus* spp., *Enterococcus faecalis*, and *Streptococcus* spp. remained detectable for up to 24 hours of exposure. Other bacterial isolates, including *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus*, and *Bacillus* spp., remained detectable for up to 48 hours. At the 48-hour exposure interval, *Aspergillus* spp. were among the fungal isolates recovered from soil, indicating continued fungal recovery during prolonged exposure under the high-temperature condition.
Microbial recovery from the other substrates was comparatively shorter. Growth was observed on the wall surface for up to 12 hours, whereas microbial recovery from the ceramic tile, glass, and floor surfaces was observed for up to 24 hours. Overall, the findings demonstrated differences in microbial persistence among substrates under the 40–45°C experimental condition.

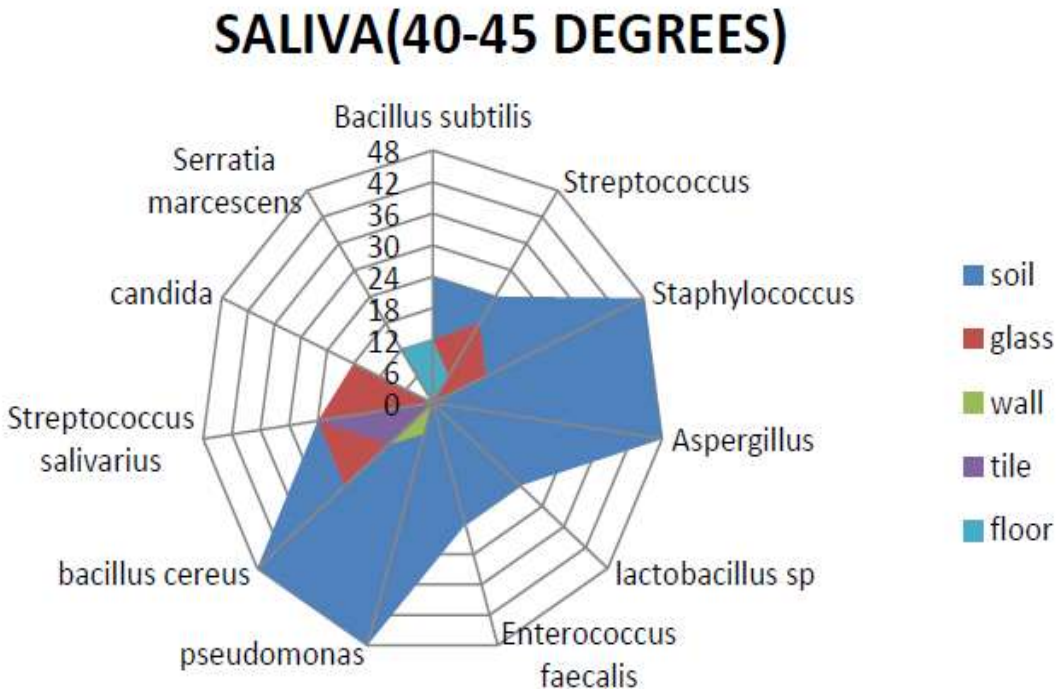


Figure 12:Distributionof*microorganisms*isolatedfrom*salivasamples*maintainedat 40–45°C across different surfaces and exposure periods.

Staphylococcus aureus,Bacillus cereus,Pseudomonas sp and *Aspergillus* were found on saliva samples upto 48 hours while *Streptococcus salivarius* was found on tile and glass surfaces upto 12 hours. Pathogenic microorganisms were able to withstand adverse surface conditions.

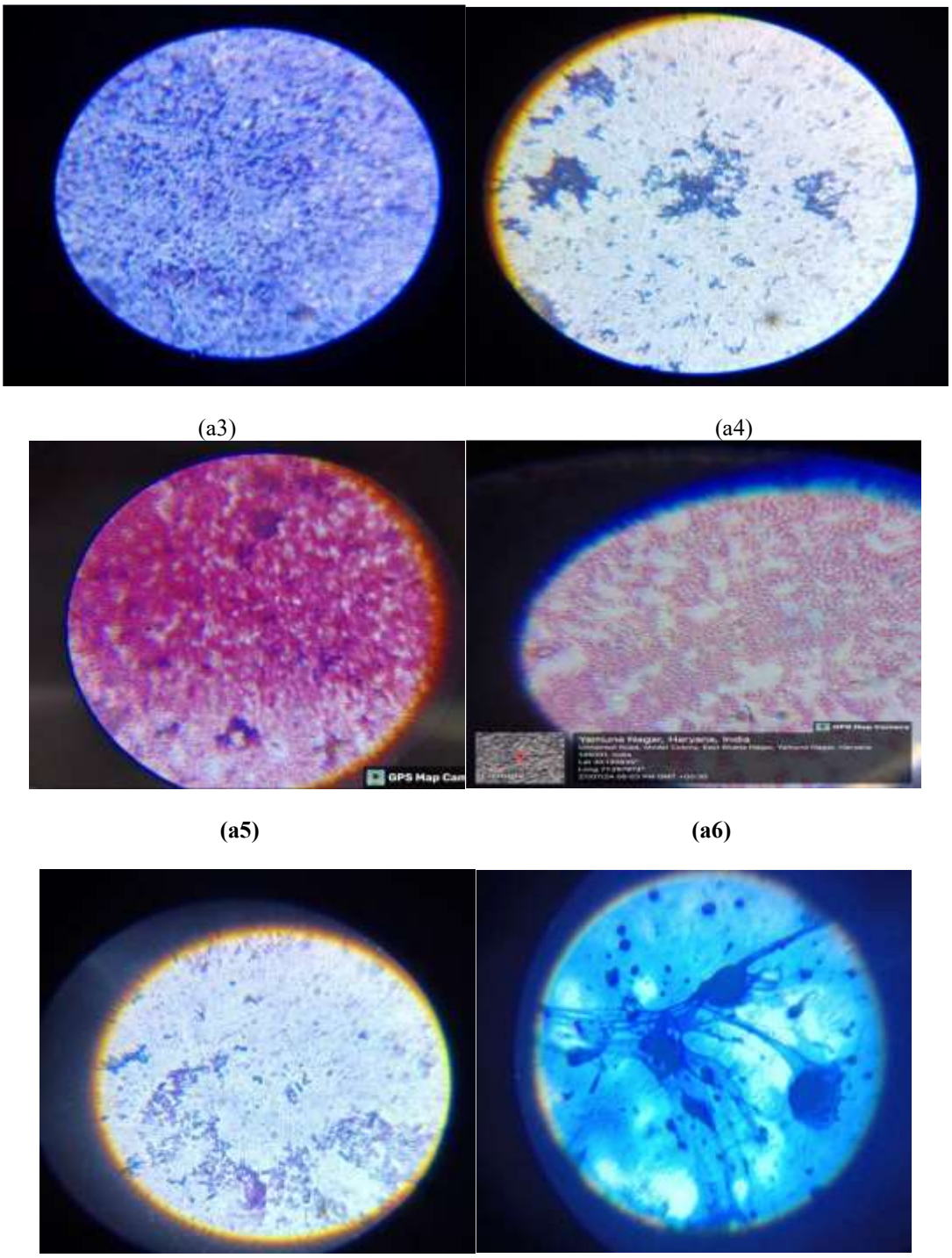


Figure 13. Representative photomicrographs of *Staphylococcus aureus(a3), Bacillus cereus (a4) from soil/floor/glass,* and *Enterococcus faecalis(a6) from soil/floor,Pseudomonas aeruginosa (a5) from soil/floor ,Bacillus subtilis(a7) from soil/glass ,Aspergillus (a8) from soil/floor surfaces,. isolated* from saliva samples maintained at 40–45°C.

Overall Observations

The overall findings demonstrated that environmental temperature, exposure duration, and substrate characteristics influenced the pattern of microbial recovery from the biological fluids examined. Among all the substrates tested, soil consistently exhibited the greatest microbial diversity across the three experimental temperature ranges of 4–9°C, 20–25°C, and 40–45°C.

Under low-temperature conditions (4–9°C), microbial recovery was comparatively limited on most non-porous substrates, including glass, wall, and ceramic tile, although selected microorganisms remained detectable during the early exposure periods. In contrast, ambient-temperature conditions (20–25°C) were associated with greater microbial proliferation and recovery across several substrates, particularly during the intermediate exposure periods. At high temperatures (40–45°C), microbial recovery varied considerably according to substrate and exposure duration, with soil continuing to support a comparatively diverse microbial population.
Overall, the results indicate that microbial persistence in blood and saliva was not uniform across environmental conditions. Rather, the observed recovery patterns varied with the **interaction of temperature, substrate type, and duration of exposure**. Soil consistently supported greater microbial diversity than the comparatively non-porous surfaces examined, whereas glass, wall, floor, and ceramic tile generally showed shorter periods of detectable microbial recovery. These observations provide a comparative basis for assessing the environmental stability and potential microbial degradation of biological fluids relevant to forensic evidence.

DISCUSSION

The preservation of biological evidence is a critical component of forensic investigation because biological fluids begin to undergo environmental alteration immediately after deposition at a crime scene. Changes occurring after deposition may influence the biological, biochemical, and microbiological characteristics of the evidence and consequently affect its subsequent forensic examination (Nasir et al., 2026). The present study investigated the microbial degradation of blood and saliva under different temperature conditions, substrates, and exposure periods. Overall, the findings demonstrate that temperature, substrate characteristics, and duration of environmental exposure interact to influence microbial recovery and the persistence of biological fluids. These findings support the need to consider the environmental history of biological evidence when evaluating its condition and forensic significance.

Influence of Temperature on Microbial Degradation

Temperature emerged as an important environmental factor affecting microbial recovery from blood and saliva. Compared with the low-temperature condition (4–9°C), the ambient-temperature condition (20–25°C) generally demonstrated greater microbial recovery across several substrates, particularly during the intermediate exposure periods. This pattern is consistent with the established principle that temperature influences microbial metabolic activity, growth, and survival (Bisker et al., 2021; Iancu et al., 2024). Under favourable temperature conditions, microorganisms may utilize the organic constituents present in biological fluids and subsequently contribute to progressive alteration of the deposited material.
The comparatively lower microbial recovery observed at 4–9°C suggests that refrigerated conditions may suppress the proliferation of many microorganisms. However, the findings also demonstrate that low temperature does not completely prevent microbial persistence. Selected bacterial and fungal organisms remained recoverable during the experimental exposure periods, particularly on soil and certain other substrates. Therefore, refrigeration may delay microbial proliferation but should not be interpreted as completely eliminating the possibility of biological degradation.
The 40–45°C condition produced a different microbial recovery pattern. Although several microorganisms remained detectable for extended periods on soil, recovery from glass, wall, floor, and ceramic tile was generally more restricted. This indicates that microbial persistence at elevated temperatures may depend strongly on substrate characteristics and the tolerance of individual microorganisms. Thus, the relationship between temperature and degradation cannot be interpreted solely as a linear increase or decrease in microbial growth.

Influence of Surface Characteristics

Substrate characteristics were another important determinant of microbial recovery. Soil consistently demonstrated the greatest microbial diversity across the three temperature conditions. This observation may be related to the naturally complex microbial community associated with soil and its capacity to retain moisture and organic material. When blood or saliva is deposited on soil, the biological material may provide additional nutrients that support microbial colonization.
In contrast, comparatively limited microbial recovery was observed on non-porous substrates such as glass, wall, and ceramic tile, particularly during the later exposure periods. These surfaces generally provide less opportunity for microbial retention and moisture preservation than soil. The findings are therefore consistent with the proposition that substrate characteristics can modify the microenvironment surrounding deposited biological material and consequently influence microbial persistence (Ogdur et al., 2018; Verdier et al., 2014).
An important observation from the present study was that the same biological fluid did not exhibit identical microbial recovery patterns across different surfaces. This suggests that environmental assessment of forensic biological evidence should consider not only the biological fluid itself but also the substrate on which it has been deposited. The substrate may influence microbial attachment, moisture availability, nutrient accessibility, and the physical stability of the biological stain.

Influence of Exposure Duration

Exposure duration also influenced microbial recovery. During the early exposure periods, microbial recovery was comparatively limited on several substrates. With increasing exposure duration, microbial colonization became more evident, particularly under ambient-temperature conditions. Greater microbial recovery was observed during the intermediate exposure periods on several surfaces, indicating that sufficient exposure time allowed microorganisms to establish and proliferate within the deposited biological material.

However, prolonged exposure did not necessarily result in continuously increasing microbial recovery on every substrate. On several non-porous surfaces, microbial recovery decreased during the later exposure periods. One possible explanation is progressive drying of the biological stain, which may reduce moisture availability and consequently limit the continued proliferation or recovery of some microorganisms. This observation emphasizes that microbial degradation is a dynamic process in which microbial growth and environmental changes occur simultaneously.

The findings are broadly consistent with previous research indicating that prolonged environmental exposure can alter biological evidence and progressively influence its forensic condition (Wahab et al., 2025). Consequently, exposure duration should be regarded as an important component of the environmental history of a biological stain.

Microbial Profiles Associated with Blood and Saliva

The study also demonstrated differences in the microbial profiles recovered from blood and saliva. Blood samples were frequently associated with Gram-positive microorganisms, while both Gram-positive and Gram-negative organisms were recovered under different experimental conditions. Soil demonstrated particularly broad microbial diversity, including organisms such as *Flavobacterium*, *Bacillus*, *Pseudomonas*, and other bacterial groups.

Among the blood-associated microorganisms, haemolytic *Streptococcus* spp. were repeatedly recovered, particularly during later stages of exposure under ambient conditions. Their recovery is relevant because microbial activity may contribute to progressive changes in the biochemical composition of blood stains. Previous research has similarly highlighted the role of microbial colonization in the alteration of biological stains during environmental exposure**.**

Saliva demonstrated a similarly dynamic microbial profile, with recovery of organisms including *Pseudomonas*, *Lactobacillus*, *Staphylococcus*, *Candida*, *Nocardia*, and other microbial groups under different experimental conditions. Previous research has reported that prolonged microbial activity can alter saliva-associated biochemical components, including detectable amylase activity (Bagban et al., 2019). Therefore, the present observations support the broader concept that microbial colonization may progressively modify the biochemical characteristics of saliva following deposition.

The recovery of different microbial populations at different exposure intervals also suggests that the microbial community associated with a biological stain may change over time. The transition from early to later microbial populations is important from a forensic perspective because the microbial profile of a recovered stain may reflect not only the original biological material but also subsequent environmental exposure.

Forensic Implications

From a forensic perspective, the findings emphasize the importance of timely recovery and appropriate preservation of biological evidence. Blood and saliva deposited at crime scenes are exposed to environmental conditions immediately following deposition, and microbial activity may progressively modify their physical and biochemical characteristics. Delayed recovery may therefore increase the possibility of microbial alteration before laboratory examination.

The implications are particularly relevant for forensic analyses involving DNA profiling, body-fluid identification, serological examination, and biochemical testing. Although the present study did not directly quantify the effect of microbial degradation on DNA profiling success, the observed changes in microbial recovery demonstrate that biological stains are dynamic environmental materials rather than chemically static evidence.

Accordingly, crime-scene personnel should prioritize rapid collection, appropriate packaging, prevention of additional contamination, and suitable preservation of biological evidence. The environmental conditions under which evidence was deposited should also be documented wherever possible because temperature, substrate, and exposure duration may contribute to the condition of the recovered material.

Theoretical Interpretation

The findings can be interpreted through an environmental degradation perspective, in which the persistence of biological evidence is understood as the outcome of interactions among the biological characteristics of the fluid and its surrounding environment. In the present study, temperature influenced the environmental conditions available for microbial activity, substrate characteristics influenced microbial attachment and the availability of moisture and nutrients, and exposure duration determined the period available for microbial colonization and subsequent alteration.

This integrated perspective helps explain why no single environmental factor adequately describes the degradation behaviour of blood and saliva. For example, soil consistently demonstrated greater microbial diversity, but the

degree and duration of microbial recovery varied according to temperature and exposure period. Similarly, non-porous surfaces generally demonstrated shorter periods of microbial recovery, but selected microorganisms remained detectable under particular temperature conditions. The results therefore support an interaction-based understanding of biological-fluid degradation rather than attributing degradation to temperature, surface, or time independently.

Limitations of the Study

Several limitations should be considered when interpreting the findings. First, the study examined only three temperature ranges and five substrate types under controlled experimental conditions. Actual crime scenes may involve substantial variation in temperature and other environmental parameters. Factors such as relative humidity, ultraviolet radiation, precipitation, airflow, seasonal variation, and repeated wetting and drying cycles were not incorporated into the experimental design and may influence microbial colonization and biological-fluid degradation.

Second, the investigation was restricted to blood and saliva. Other biologically relevant forensic materials, including semen, vaginal secretions, urine, and tissue material, may demonstrate different microbial and environmental degradation patterns. Third, the study used samples from five healthy adult volunteers; consequently, biological variation among individuals and differences in the composition of biological fluids may not be fully represented.

Finally, the present study primarily assessed microbial recovery and did not directly quantify the extent to which the observed microbial activity affected DNA quantity, DNA quality, or the success of downstream forensic identification. Future studies should therefore integrate microbiological analysis with molecular and biochemical assessments to establish more direct relationships between microbial degradation and forensic examination outcomes.

Future Research and Overall Interpretation

Despite these limitations, the study provides experimental evidence that temperature, substrate characteristics, and exposure duration jointly influence microbial recovery from blood and saliva. The consistent diversity observed in soil and the comparatively shorter microbial persistence on several non-porous surfaces demonstrate that the environmental context of a biological stain is an important consideration in forensic evidence preservation. Future investigations should incorporate a wider range of environmental variables and longer observation periods and should combine microbiological methods with DNA quantification, DNA integrity assessment, body-fluid identification markers, and biochemical assays. Such integrated research could provide a stronger understanding of the relationship between microbial colonization and the actual loss of forensic evidential value.

Overall, the findings indicate that biological fluids cannot be considered environmentally stable following deposition at a crime scene. Their microbial profile and physical and biochemical condition may change progressively according to the interaction of temperature, substrate, exposure duration, and microbial activity. The study therefore reinforces the forensic importance of rapid evidence recovery, appropriate preservation, and careful consideration of environmental exposure when interpreting biological evidence.

CONCLUSION

The present study demonstrates that the microbial persistence and degradation of forensic biological fluids are influenced by the combined effects of environmental temperature, substrate characteristics, and duration of exposure. By examining blood and saliva deposited on five commonly encountered inanimate substrates—soil, glass, wall, floor, and ceramic tile—under three temperature conditions (4–9°C, 20–25°C, and 40–45°C) and across exposure periods of 6–48 hours, the study provides experimental evidence of differences in microbial recovery and persistence under varying environmental conditions. These findings highlight the importance of considering the environmental history of biological evidence when assessing its condition and potential forensic value.

Temperature was an important determinant of microbial recovery. Ambient conditions (20–25°C) generally supported greater microbial proliferation across several substrates, particularly during the intermediate exposure periods, whereas the low-temperature condition (4–9°C) was associated with comparatively limited microbial recovery on several non-porous surfaces. However, selected microorganisms remained detectable even under low-temperature conditions, demonstrating that refrigeration may suppress microbial proliferation rather than completely prevent microbial persistence. At 40–45°C, microbial recovery varied according to substrate and exposure duration, with soil continuing to support diverse microbial populations while recovery from several non-porous surfaces was comparatively shorter. These findings indicate that the effect of temperature on biological-fluid preservation is dependent on the interaction between environmental conditions and substrate characteristics.

Substrate characteristics also played an important role in microbial persistence. Soil consistently demonstrated the greatest microbial diversity across the three temperature ranges. Its naturally complex microbial community and capacity to retain moisture and organic material may provide favourable conditions for microbial colonization of deposited biological fluids. In contrast, glass, wall, ceramic tile, and floor surfaces generally demonstrated

shorter periods of detectable microbial recovery, particularly under low-temperature conditions. These findings indicate that the substrate on which biological evidence is deposited should be considered when evaluating its potential exposure to microbial degradation.

Exposure duration further influenced microbial recovery. Microbial presence was generally more limited during the early exposure periods and became more evident with increasing exposure time, particularly under ambient-temperature conditions. The intermediate exposure periods, especially 12–24 hours, demonstrated comparatively greater microbial recovery across several experimental conditions. Although microbial recovery subsequently decreased on some non-porous surfaces, this reduction should not necessarily be interpreted as the absence of prior degradation, as progressive drying and changes in the biological stain may influence subsequent microbial recovery. The findings therefore emphasize the importance of minimizing delays between crime-scene deposition, evidence recovery, and laboratory examination.

The study also demonstrated distinct microbial profiles associated with blood and saliva. Blood samples were frequently associated with Gram-positive microorganisms, while both Gram-positive and Gram-negative bacteria were recovered under different environmental conditions. Soil supported a particularly diverse microbial population, including organisms such as *Bacillus*, *Pseudomonas*, *Flavobacterium*, and other bacterial groups. Haemolytic *Streptococcus* spp. were also recovered from blood samples, particularly during later stages of exposure under ambient conditions. Saliva samples similarly demonstrated changes in microbial recovery over time, with organisms including *Pseudomonas*, *Lactobacillus*, *Staphylococcus*, *Candida*, and *Nocardia* spp. identified under different experimental conditions. These observations demonstrate that the microbial profile associated with a biological stain may change during environmental exposure.

From a practical forensic perspective, the findings emphasize the importance of rapid evidence recovery, appropriate packaging, contamination control, and suitable preservation. Biological evidence deposited on soil may require particular attention because of the comparatively high microbial diversity observed on this substrate. Likewise, evidence exposed to favourable environmental temperatures may undergo more rapid microbial alteration. Knowledge of the substrate, environmental temperature, and approximate duration of exposure may therefore assist forensic personnel in assessing the condition of recovered biological evidence and interpreting subsequent laboratory findings.

Growth at 20-25^{0}C>Growth at 40-45^{0}C>Growth at 4-9^{0}C.

The study has several limitations. The investigation was conducted under controlled laboratory conditions and included only two biological fluids, five substrates, three temperature ranges, and exposure periods extending to 48 hours. Environmental variables such as relative humidity, ultraviolet radiation, rainfall, airflow, seasonal variation, and outdoor exposure were not incorporated into the experimental design. These factors may influence microbial colonization and biological-fluid degradation under actual crime-scene conditions. In addition, microorganisms were identified primarily using conventional microbiological culture, staining, and biochemical approaches rather than molecular identification methods. The findings should therefore be interpreted within the scope of the experimental conditions examined.

Future research should incorporate a broader range of environmental conditions, substrates, biological fluids, and exposure durations. Integration of conventional microbiological techniques with molecular identification, DNA quantification, DNA integrity assessment, and biochemical markers of body-fluid degradation would provide a more comprehensive understanding of the relationship between microbial activity and the loss of forensic evidential value. Field-based studies could further determine whether the patterns observed under controlled conditions are reproducible in realistic crime-scene environments.

Overall, this study provides experimental evidence that microbial degradation of blood and saliva is a dynamic process influenced by the interaction of temperature, substrate characteristics, and exposure duration. The findings address an important research gap concerning the combined influence of these environmental variables on microbial persistence in commonly encountered forensic biological fluids. The study reinforces the necessity of timely collection and appropriate preservation of biological evidence and provides a scientific basis for considering environmental exposure when evaluating the reliability and potential forensic significance of biological material recovered from crime scenes..

References

1. Aloraer, D. (2017). Evaluation of collection protocols for the recovery ofbiologicalsamples from crime scenes [University of Lancashire]. In CLOK (University of Central Lancashire).
2. Bagban, M., Parmar, N., & Jain, N. (2019). Extraction & purification of hemolysin from toxinproducingmicroorganismsanditseffecton forensically important blood and saliva samples. Bacterial Empire, 2(4), 76–76.

3. Bisker,C.,Taylor,G.,Carney,H.,&Ralebitso-Senior,T.K.(2021).ACombinedApplication of Molecular Microbial Ecology and ElementalAnalysesCanAdvancetheUnderstandingof Decomposition Dynamics. Liverpool John Moores University, 9.
4. 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.
5. Elliott, C., Shafer, A. B. A.,&Stotesbury,T.(2022).Thecruxoftime:Ameta-analysisofex vivo whole blood degradation. Frontiers in Analytical Science, 2.
6. Gracia-de-Lucas, S., López-Matayoshi, C., Lozano-García, M., & Gomes, C. (2026). Unearthingforensic genetics: preliminary results on the ability to generate DNA profiles from buried human biological samples. International Journal of Legal Medicine.
7. 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.
8. Joseph, É. (2021). Microorganisms in the Deterioration and Preservation of Cultural Heritage.
9. Lim, D. V., Simpson, J. M., Kearns, E. A., & Kramer, M.F.(2005).CurrentandDeveloping Technologies for Monitoring Agents of Bioterrorism and Biowarfare. Clinical Microbiology Reviews, 18(4), 583–607.
10. 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.
11. Ogdur, M., Çakan, H., & Çevik, F. E. (2018). Investigation of the microorganisms decaying bloody evidences. Medicine Science | International Medical Journal, 1–1.
12. Pajnič, I. Z. (2025). Analysis ofHumanDegradedDNAinForensicGenetics.Genes,16(11), 1375–1375.
13. Rankin-Turner, S., Turner, M. A., Kelly, P. F., King, R. S. P., & Reynolds, J. C. (2018). Transforming presumptive forensictesting:insituidentificationandageestimationofhuman bodily fluids. Chemical Science, 10(4), 1064–1069.
14. 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.
15. Song, Y., Huang, Y.-Y., Liu, X., Zhang, X., Ferrari, M., & Qin, L. (2014). Point-of-care technologies for molecular diagnosticsusingadropofblood.TrendsinBiotechnology,32(3), 132–139.
16. Swayambhu, M., Kümmerli, R., & Arora, N. (2023). Microbiome-Based Stain Analyses in Crime Scenes. PubMed Central, 89(1).
17. 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).
18. 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.
19. Wahab, A. M. A., Menon, S., & Jacob, A. A. (2025). Evaluation of Microbial Growth in Dried Blood Stains. 3750–3753.
20. 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,

..