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Microbial Analysis of Rabbit Skin Decomposition in Different Soil Compositions

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Abstract

This research paper aims to determine how different types of soil affect the microbial activity during decomposition. The study was conducted in a span of 28 days, and observation was done for every 7 days. Rabbit skin was chosen as a biological specimen to breakdown soft tissue decomposition under natural soil conditions. Based on their physical and chemical parameters three types of soil were selected alluvial, black, and red soil. The assessment involved measuring microbial activity and changes in the environment during decomposition which included pH measurements, bacterial staining, and fungal staining. Major differences were noted in the activity of microbes within the different types of soil, as determined from the results. Microbial activity was most abundant in alluvial soil since it is well structured and contains rich nutrients. Black soil had some amount of active microbes, while red soil had the least amount of microbial activity due to its lack of organic material and its acidic nature. This study explains how the soil types influencing decomposition and microbial succession. The results offer valuable insight for forensic science, particularly in determining postmortem interval and understanding the decomposition process in various environmental conditions.

Keywords: *Microbial activity, soil, microbial growth, soil microbiology, rabbit skin decomposition and post-mortem interval estimation.*

1. Introduction

Decomposition is one of the important processes for ecological cycling of nutrients and plays an important role in forensics. Decomposition is a complex process influenced by a myriad of microbes and environmental factors characterize [1], including the type of soil which is crucial for microbial activity pH, moisture, and organic matter break down rate. The processes that occur during decomposition, combined with the different soil types, can demonstrate the microbial successions in control succession [2], and environmental conditions as volumetric water content serves temperature gradients through varying the depth in the soil oxygen and moisture conditions [1].

The study of natural decomposition has become increasingly important in forensic examinations of time of death estimation, locating concealed graves, and interpreting postmortem interval (PMI) taphonomic changes. The important consideration within the captioned contexts lies in the fact that some tissues such as the skin decompose very fast under the influence of microbes and environmental variable changes which makes it important for forensic examination. In relation to the above presented problem, the research goals could be: To study the microbial processes associated with skin decomposition in different soils.

Our aim for this paper is to study the microbial impact of decomposing rabbit skin in alluvial, black, and red soils, chosen for marked differences in their physical and chemical composition. Nutritious, well-drained `alluvial soil`, is thought to harbor heavy microbial activity. In contrast, black and red soils may have altered decomposition effects due to their moisture laden clay structure and acidic, organic matter deficient nature, accompanied by high clay content in black soil and acidic nature coupled with low organic matter in red soil.

Microbial and environmental evaluations on the biological substrate, in this case, rabbit skin, were done on a weekly basis with bacterial and fungal staining along with pH analysis performed at the end of the 28 days duration. Other analyses were done to assess the soil environment's response to soft tissue decomposition over time.

This research aids in explaining the archaeological context of a body by focusing on soil type influences on microbial succession and decomposition strategies. The approach hopes to extend the understanding of forensic taphonomy as well as decomposition context and primary interpretational estimating frameworks for Post Mortem Intervals dominantly under various environmental conditions.

2. Methodology

To examine soil microbial activity and environmental changes related to decomposition of rabbit skin in alluvial, black, and red soil over 28 days utilizing bacterial, fungal, and pH analysis every seven days.

2.1 Skin Sample Collection: Fresh Rabbit skin samples were used as a model for soft tissue decomposition under different soil conditions. The samples were obtained from a local vendor who was registered with the appropriate authorities, ensuring that no animals were harmed for the sake of the study. The skin was removed immediately post-mortem in order to maintain the microbial ecosphere and tissues so as to not damage the natural flora [4].

2.2 Tissue Selection and Justification: Based on the presence of epidermal strata and microbial colonization patterns, rabbit skin was chosen over other tissues and full carcasses for its resemblance to humans. In forensic science, it provides an ethical and controllable method to study decomposition and microbial successions [5]. It also improves the study by providing focused examination on surface microbial relations crucial for posting grinding indications of time elapsed after death estimation. Full carcasses are more complicated due to organ decay.

2.3 Soil: For these analysis, three contrasting soil types were collected from Thenkarumbalur (12.09836°N, 79.00109°E), Tiruvannamalai (12.23363° N, 79.06687° E), Tamil Nadu, India. The three different soil types are Red soil, Black soil, and alluvial soil. These soil types were selected based on their contrasting physical and chemical characteristics [13-14].

2.4 Experimental Design: Three different soil types such as black soil, alluvial soil, and red soils were sourced from natural unblemished regions, green at underrated locations. All samples of soil contained the moisture of 60% water consistency to represent natural decomposition conditions. Fresh rabbit skin was collected from a nominated ethical source, sampler was collected from fresh sources, alongside conveyed under antiseptic conditions. The skin was chopped into equal square pieces approximately 5 X 5 cm so recon the rabbit parameters are standardized. In total, a dozen rabbit skin samples of 12 were assigned for experimentation. Each sample had the required relevant alterations boxed into individual sterile plastic containers each filled with one of the 3 selected types of soils and placed at around 10cm below surface or soil level. 4 sets per containing 4 containers were prepared leading to an interchangeable triplet pattern for all soil types to capture individual inspection on day seven, fourteen, twenty, and twenty-eight with enough for reliability alongside diversity. Throughout the study, the box was kept at room temperature 25-28°C alongside during temperature aligning phase. Mid project incorporating these alterations enables further refinement of the exploration's conclusions. Region supplying soils devoid of blemishing allows the study calibration elation against modifying environmental during material training.

2.5 Periodic Sample Recovery: One replicate of each soil type was meticulously excavated using sterile instruments at seven-day intervals. Before being ready for microbial analysis, the skin was gently cleaned to get rid of extra dirt.

2.6 Microbial Analysis Techniques: Microbial growth on the decomposing rabbit skin were analysed using bacteria and fungi microbiological staining methods. These techniques help to identify the microorganisms present on their characteristics and morphology.

2.7 Bacterial Staining: Surface material from the rabbit skin was collected using inoculation loop to create smears on the pristine glass slides. Widely used Gram's staining method were used to identify the structure of the cell wall to distinguish between Gram-positive and Gram-negative bacteria. After staining the glass slides were examined under a light microscope at 1000x with immersion oil. Bacterial types were identified and classified by their shape (Cocci or Rods) and staining colour (pink for Gram-negative, pink for Gram-positive).

2.8 Fungal Staining: Small portions of Rabbit skin were mounted on glass slides with blue ink and vinegar for fungal identification. Which fungal structures including fungal hyphae, and spores. Compound microscope were used to identify fungal developmental patterns and differentiate between different types of fungal colonies based on their appearances.

2.9 pH Analysis of Soil: The pH of the soil was determined by making 1:2.5 soil-water suspension for each interval. The suspension was thoroughly stirred before being allowed to settle. A calibrated digital pH meter was used to measure the pH.

2.10 Scoring Scale for Bacterial and Fungal Activity Intensity: This table provides bacterial and fungal scores based on the activity intensity in rabbit skin samples during analysis intervals.

Table 1. Scoring scale for bacterial and fungal activity intensity.

Sr. No.	Bacterial and Fungal activity	Score(s)
1	No activity	0
2	Very few activity	1

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3	Activity in several fields	2
4	Moderate activity	3
5	Dense activity	4
6	Extremely dense activity	5

2.11 Graphical Analysis: To visually interpret the microbial activity and soil environment changes over time, Microsoft Excel were used to create line graphs for all parameters such as bacterial growth, fungal growth, and soil pH.

For each parameter, the values recorded at 7-day intervals (day 0, 7, 14, 21, and 28) were plotted on the graphs.

3. Results

3.1 Bacterial Staining: A line graph study of bacterial growth over the 28 days observation period indicated significant variations in microbial activity among the three soil types: red, alluvial, and black. The microscopic examination across soil types revealed various microbial succession of various bacterial communities such as Bacilli, Cocci, Sparse Bacilli, and Psuedomonas spp. Bacterial growth gradually increased in red soil, peaked at Day 21 with the bacterial load score of 4, and then decreased by day 28 [6]. Due to this pattern, red soil may provide a favorable environment for bacterial growth during the intermediate stages of decomposition, but it may become less favorable as the observation period completes. In alluvial soil, bacterial load peaked earlier with the score of 3 at day 14, with the score of 3. A consistent fall followed, suggesting that microbial activity in this soil began and ended faster, potentially due to its finer texture or nutrient concentration, which may lead to faster microbial responses [11]. Throughout the whole study, black soil showed minimal bacterial activity. Low and steady load of 1 was observed at day 7, followed by complete reduction to 0 at day 14, showing that this soil type may either prevent bacterial growth or breakdown tissue in an environment that is more resistant to bacterial colonization.

Overall, bacterial growth trend indicate that red and alluvial soils have different microbial schedules, but black soil has limited replication of bacteria [12]. These variations indicate the role of soil elements on microbial dynamics during decomposition.

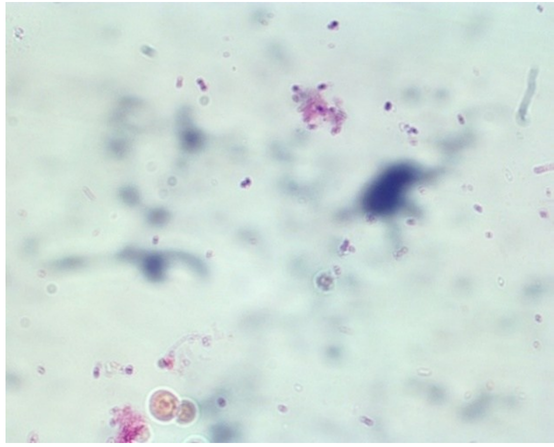

Figure 1. Bacterial growth activity – Score "0".

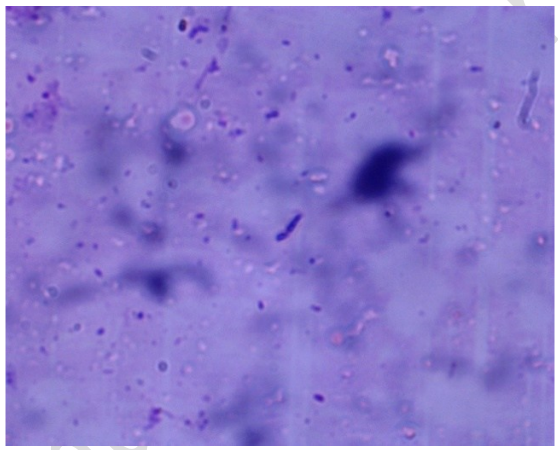

Figure 2. Bacterial growth activity – Score "1".

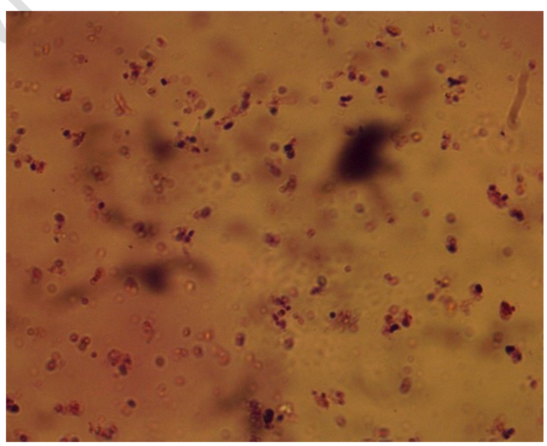

Figure 3. Bacterial growth activity – Score "2".

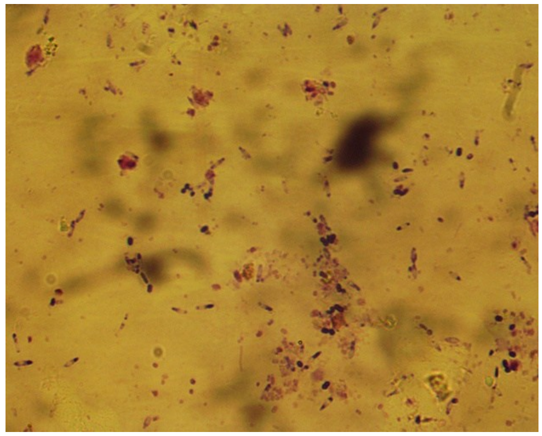

Figure 4. Bacterial growth activity – Score “3”.

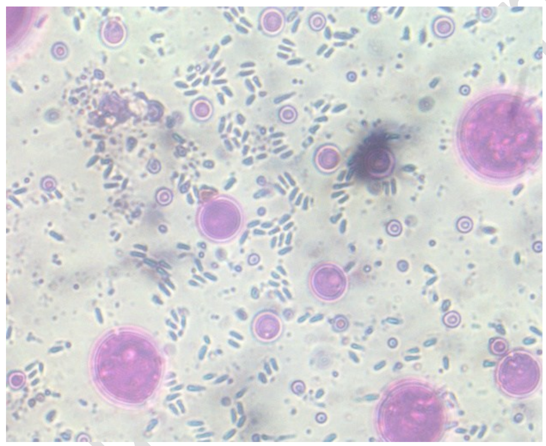

Figure 5. Bacterial growth activity – Score “4”.

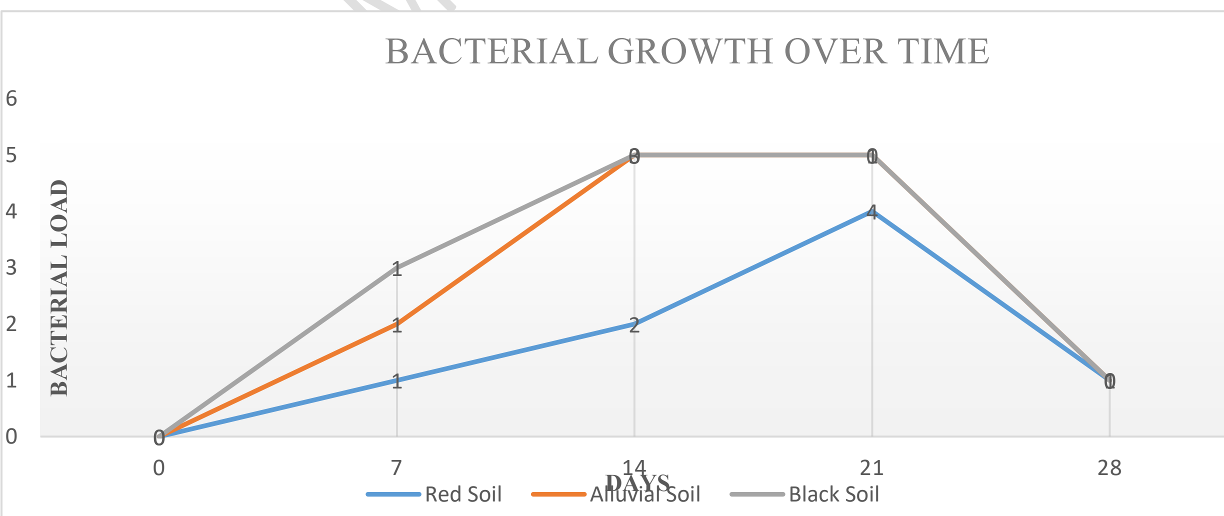

Figure 6. Line graph representing bacterial load observed on rabbit skin buried in red, alluvial, and black soils over 28 days.

3.2 Fungal Staining: The development of fungal colonization on decomposing rabbit skin revealed different pattern in each soil type, as shown as line graphs. The microscopic examination across soil types revealed various microbial succession of various fungal communities such as Penicillium spp, Aspergillus spp, Fusarium spp, Candida spp, and Cladosporium spp. Fungal activity in red soil was absent on days 7, 21, 28, and only showed a significant increase on day 14. Fungal growth in red soil may have been affected by an unexpected window of favorable conditions, probably related to temporary moisture or pH changes, as indicated by this sudden, separated an increase followed by a sharp decline. In contrast, alluvial soil showed a steady fungal load between days 7 and 21, resulting at a stable score of 5. This soil type appears to sustain long-term fungal colonization, most likely due to its finer texture and ability to retain organic matter and moisture-conditions that promote fungal durability. A drop to zero by day 28 may indicate a natural decline in available nutrients as decomposition took place. In black soil, fungal activity began strongly on day 7 and stayed constant until day 28, with final rise again observed at day 28. This pattern indicates that continuous fungal colonization throughout the decomposition process that may be because of its mineral richness and greater initial pH.

Overall, the fungal growth pattern shows that while all soil types supported fungal activities to different degrees, alluvial and black soil were more favorable environments for continuous fungal colonization than red soil, which showed a short, isolated peak in activity.

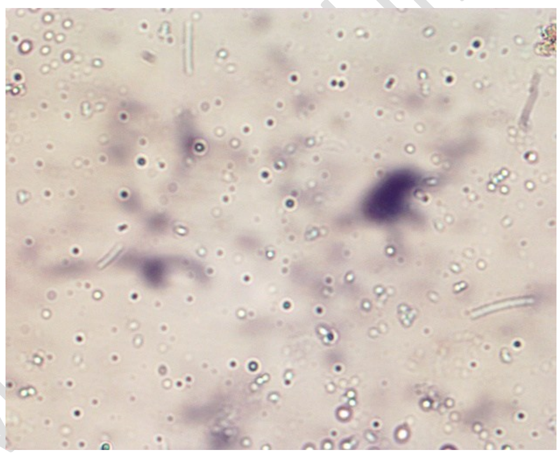

Figure 7. Fungal growth activity – Score "0".

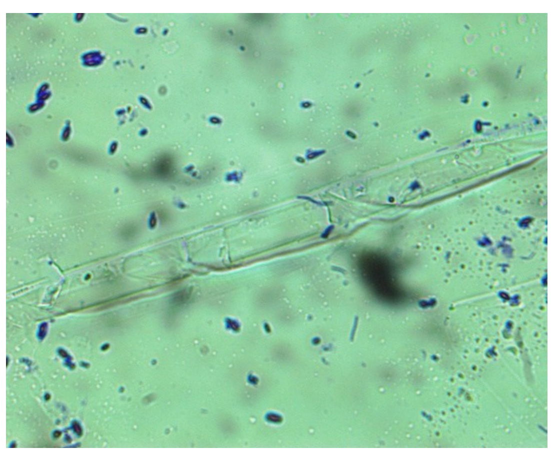

Figure 8. Fungal growth activity – Score "1".

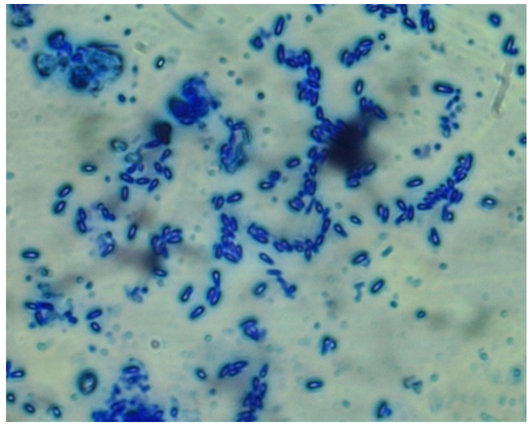

Figure 9. Fungal growth activity – Score "3".

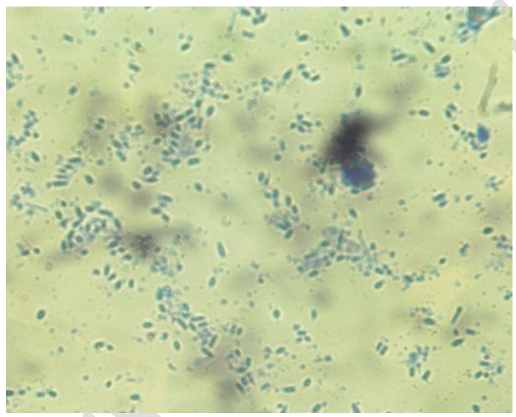

Figure 10. Fungal growth activity – Score "4".

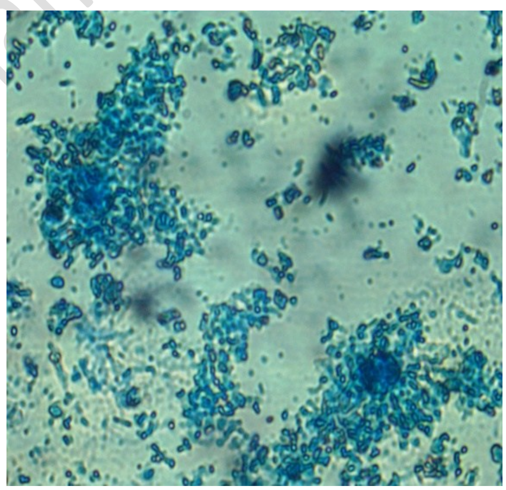

Figure 11. Fungal growth activity – Score "5".

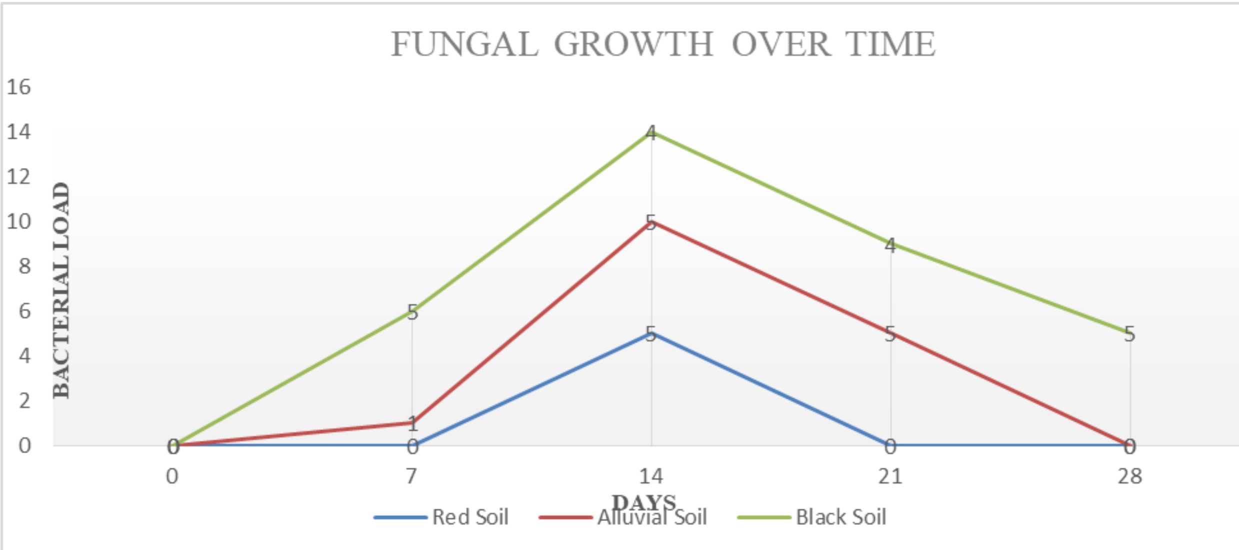

Figure 12. Line graph representing fungal load observed on rabbit skin buried in red, alluvial, and black soils over 28 days.

3.3 pH Analysis: The pH values observed over the 28 days period provided that how rabbit skin burial and decomposition affected the surrounding soil ecosystem. Each soil revealed an individual pH fluctuation pattern, indicating variations in chemical composition and microbial interactions [19]. The pH in red soil decreased significantly from 8.5 on day 0 to 6.2 on day 21, indicating that acidity increased during active decomposition. This decrease corresponds to the peak in bacterial activity recorded on day 21, which possibly influenced the acidity through metabolic by parties [10]. However, on day 28, the pH had slightly increased to 7.2, probably indicating the start of environmental recovery or stabilization. Throughout the study, alluvial soil maintained a reasonably steady alkaline pH of 8.7 to 9.2. The constant pH, even during active microbial development, indicates that this soil type has suitable buffering capability. This stability may explain the continuous fungal activity observed in this environment as some fungal species survive in slightly alkaline environments [15]. In contrast, black soil started with highest pH 9.9 on day 0 and showed only slight changes and decreasing to 8.6 on day 7, then stabilizing around 9.1 before dropping again slightly at the end. Despite these changes, black soil remained strongly alkaline throughout the study. This adequacy might result in the low bacterial proliferation and continuous presence of fungi. These patterns collectively illustrate that decomposition processes alter soil chemistry differently based on the type of soil. For example, red soil became more acidic during decomposition, while alluvial and black soils remained alkaline. This change likely affected the type and level of soil microbial attachment.

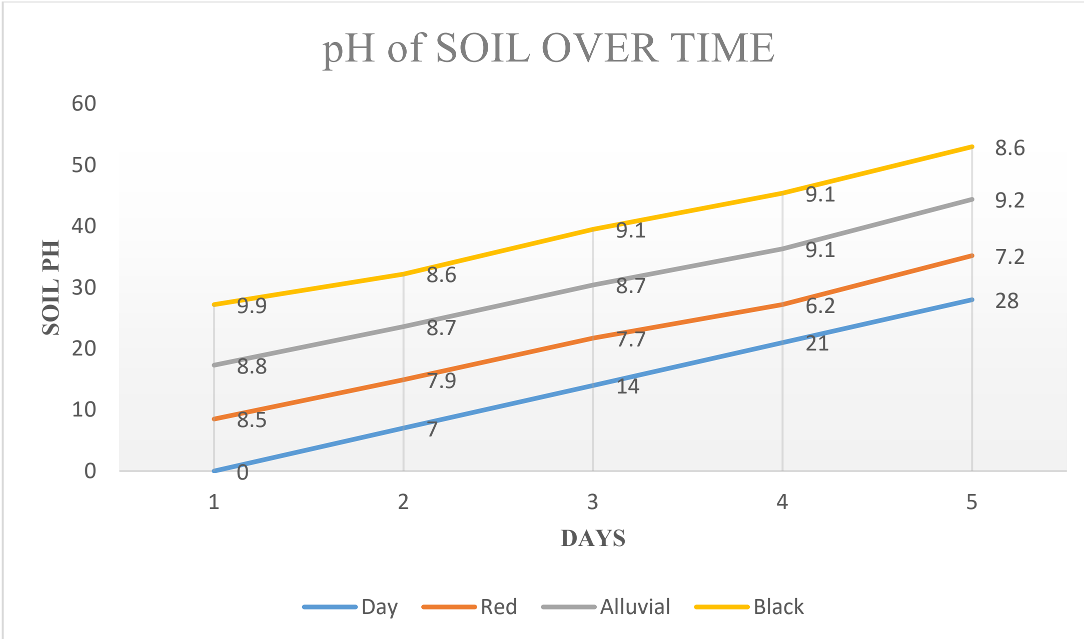

Figure 13. Line graph representing soil pH variation in red, alluvial, and black soils.

4. Discussion

To achieve this goal, the study aimed to assess the level of soil microbial activity related to the decomposition of rabbit skin buried in red, alluvial, and black soils during a 28-day period. Changes in bacterial and fungal measurements, along with the pH of the soil, were monitored in order to analyze how decomposition and microbial colonization were influenced by the type of soil.

The changes in the type of soil in combination with the amount of bacteria present yielded the most diverse results. During the course of this study, red soil showed the most bacteria activity peaking on day 21. This might have been because of the slightly acidic to neutral pH and high organic content that appeared to aid in supporting bacterial growth. On the contrary, black soil did not contain any bacteria throughout the duration of the study, suggesting that there are likely more alkaline elements alongside certain suppressive minerals that inhibit bacterial growth in the soil. There were also signs of bacterial activity in alluvial soil which was noted to be visible around day 14 [3].

In terms of fungal activities, black soil exhibited the highest and most consistent fungal activity throughout the 28 days, with the highest level of activity at the end of the study. This is consistent with a functioning life cycle of certain fungi that thrive in alkaline or mineral rich areas as some black soils do contain [7]. Alluvial soil supported moderate fungal activity, especially in the middle phase from day 14 to 21, while red soil exhibited fungal activity only on day 14 and day 21. The low level of fungal activity in red soil may indicate less favorable conditions or heightened demand by bacteria during peak metabolism.

The pH analysis supplements the microbial data. Red soil had the greatest fluctuation of pH with the highest of 8.5 and lowest of 6.2 on day 21, which was mostly its own microbial activity and the release of organic acids due to microbial degradation. While remaining mostly alkaline, alluvial and black soils, black soil still relatively stable, showed more decline. What these results suggest is that the decomposition profoundly modifies the soil chemistry [9], but the other way decomposition modifies the soil properties, profoundly affects microbes succession.

The results of this research suggest that the type of soil is an important factor in the regulation of microbial activity during degradation of soft tissue. Red soil supports active bacteria and pronounced chemical change, while black soil supports fungal persistence with little pH change [18]. Alluvial soil reacts the most neutral to the two biological factors we tested. Knowing the microbial composition with respect to time and soil is important in forensic science and especially in estimating time of death based on the condition of the remains in relation to the soil they decompose in.
The study concludes that the type of soil significantly affects the level of microbial activity during the decomposition of soft tissue. Further results revealed that red soil has more active and volatile bacteria and undergoes greater chemical changes compared to black soil which is dominated by fungi and experiences very little change in acidity. Alluvial soil was also found to be more active in relation to his hypothesis. These relationships are essential to understand, especially in forensic science where the time of death has to be determined

5. Conclusion

The soil type has a crucial influence on the microbial decomposition of rabbit skin, especially regarding the activity of bacteria, fungi, and the soil's pH over time. The greatest microbial activity as well as substantial pH swings indicative of strong microbial activity during decomposition was observed in red soil. Black soil supported more or less constant development of fungi, while alluvial soil had an intermediate level of bacterial and fungal activity. With regard to forensic aspects, these results emphasize the need for studying soil variables in relation to changes in decomposition. This evidence suggests that the pattern of microbial activity and the change in soil constituents could assist in estimating the post-mortem interval (PMI) for remains with soft tissues that are buried. Adding quantitative microbiological analyses along with extended timelines would enhance the forensic application of the soil.

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