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Effects of soil and water on succession of thanatomicrobiome and estimation of postmortem interval

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ABSTRACT:

Studies in forensic microbiology have examined the thanatomicrobiome as a new technique for determining the postmortem interval. This study focuses on the impact of various kind of soil, and water on internal organs of body after death as the bodies can be found buried under soil, and in water. Thus, this study was performed to characterise the thanatomicrobiome signatures of victim's internal organs, such as the liver and kidney, in cadavers. Autolysis and putrefaction are two postmortem processes that are impacted by a variety of variables, including soil characteristics, microbial activity and humidity. Determining the microbial succession in water and soil at various intervals of time and at various temperatures was the aim of this investigation. The outcomes of the soil analysis and the decomposition were found to be in agreement. The current study concluded that location of the crime scene can also be determined using the post mortem interval and a thorough knowledge of decomposition procedure with respect to the microbes growing on cadaver. PMI estimation can also be enhanced by studying microbial succession based postmortem submersion interval (PMSI) considering the importance of soil and water quality. Future models of bacterial communities associated with postmortem microbial assemblage in internal organs can be created using this impact.

Keywords: Thanatomicrobiome, Decomposition, Forensic Science, Post-Mortem Interval, Post-Mortem Submersion interval, Succession.

INTRODUCTION:

Decomposition is a postmortem procedure that includes putrefaction (Love, et al., 2003) and autolysis is used to calculate the postmortem interval (PMI) along with a number of variables. Even though forensic science has studied decomposition extensively, still determining time since death remained an area which has not received much attention. Numerous research on the effects of several elements on decomposition has already been carried out including body size, humidity (Garriga, et al., 2017), temperature (Archer, 2004), Microbiological activity (Tibbett, et al., 2004) conditions, soil pH, and wetness (Brown, 2003, Haslam, et al., 2009, Micozzi, et al., 1997, Sunagawa, et. al., 2013) where the PMI of carcass was established. Soil's bio physicochemical qualities have been utilised as a sign of criminal activity (e.g., the deposition of a body), its

chemical and biological characteristics also investigated in order to calculate PMI (Love, et al., 2003, Ayers, 2010, Carter, 2005**,** Vass, et al., 1992,2001) and locating clandestine graves (Carter, 2003, Rodriguez, et al., 1985).
It is commonly recognised that the size of the soil particles directly influences the breakdown of organic content in an organism and as a result gases diffuse through the soil matrix (Tibbett, et al., 2004, Fiedler, et al., 2003, Johnson et al., 2019, Santarsiero, et al., 2000). Decomposition in soil that is coarse-textured can be either totally blocked or greatly reduced (Tibbett, etal., 2004, Fiedler, etal., 2003, Johnson etal., 2019, Santarsiero, etal., 2000). In contrast, the presence of elevated levels of calcium, phosphorus, and manganese (Bethell,1987) in coarse-textured, high moisture soil may lead to the formation of pseudomorph. Anaerobes, which are less effective decomposers (Swift, etal., 1979), dominate fine-textured (clayey) soil because it has a lower rate of gas diffusion than coarse-textured soil. As a result, oxygen-CO_2 exchange may not be sufficient to produce aerobic microorganisms. These can cause a reduction in gas exchange, which can slow down decomposition (Hopkins, etal., 2000, Turner, etal., 1999). Moreover, adipocere development can be seen around the cadaver or inside the organs. However, as there doesn't seem to have any research on how soil texture affects decomposition, the information stated above is accepted and expected based on tests.

Bodies retrieved from water pose difficulties to the investigating authorities, particularly corpses at an advanced stage of decay. The cause of death and postmortem submersion interval (PMSI) are often determined by a forensic pathologist.Where death has taken place due to submerging of bodies, the PMSI is a useful measure to determine time since death. Interior microbial community succession of water-submerged corpses was estimated and explored on how this might be applied to forensic investigations to provide precise PMSI estimates (Zhang, et al., 2022).

This work focuses on estimating PMI by determining the microbial diversity and species abundance in distinct postmortem liver and kidney samples from animal models, at different times and temperatures, and under the effect of soil, and water (Vass, et al., 1992; 2001).

Estimation of amount of time after death can be aided by the time-dependent changes in the thanatomicrobiome within the internal organs. According to studies conducted by (Hyde, et al., 2013, 2015, Tuomisto, et al., 2014, Can, et al., 2014, Yuan, et al., 2022, Damann, et al., 2015, and Hauther, et al., 2015, Hewadikaram, et al., 1991, Lynne, et al., 2015), it was possible to find thanatomicrobiomes in internal organs and cavities after death. After death, temperature and duration affect the qualitative and quantitative composition of microbiota in internal organs, also there is a shift in microbiota basically from simple and unstable form to complex and stable form (Lawrence, etal., 2019)**.**

The culture-independent method entails the direct extraction and sequencing of genetic material from the dead and decomposed samples, and as a result, it represents a more accurate, thorough, and high-throughput profile of the isolated microbiota (Lagier, et al., 2015; 2016). Using this technique, a genomic region that is variable between taxa but conserved within a taxon is amplified before the marker gene is sequenced. Common marker genes have been used, such the 16sRNA gene for bacteria and the internal transcribed spacer for yeast and fungi.

MATERIALS AND METHODOLOGY:

Fresh liver and kidney samples of goat (Fig. 1) were collected from the butcher shop. The material was then stored in two environments viz, soil and water in both anaerobic (by using zip locks) and aerobic conditions (Fig. 2), and then exposed to a range of temperatures (37°C, 27°C, and 4°C) for testing at various intervals of time (3hrs., 24hrs., 48hrs. and 72hrs.).
Saline solution was used to make the extract at each time interval (Fig. 3). The agar medium (Fig. 4) was autoclaved for 45 minutes at 115°C. The culture was prepared by spreading the extract over the agar plates, and was then incubated at different temperatures. The plates were observed for growth (Fig.8) and potential microbial colony isolation was done at every 24 hours.

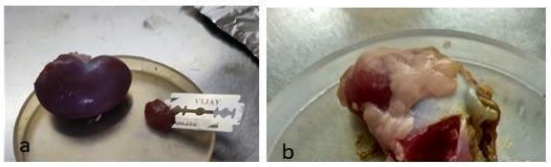

Fig.1 (a) Liver and (b) Kidney samples


Fig.2 Sample Preparation in soil and water in both aerobic and anaerobic condition


Fig.3 Extract Preparation


Fig.4 Nutrient agar media


Fig.5 Anaerobic candle jar


Fig.6 Maggots formation after 48hours of incubation

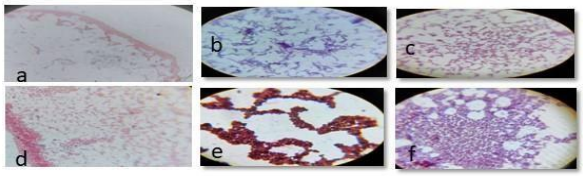

Fig.7 Gram Staining results
(a) Short rods –ve, (b) Rods +ve, (c) Cocii +ve, (d) Short rods –ve, (e) Yeast cells and (f) Gram +ve rods

Fig.8 Growth of colonies at different time intervals
(a) after 24hrs., (b) after 48hrs., and (c) after 72hrs.

Gram staining (Fig.7) was carried out to separate pure colonies for additional analysis.
Biochemical tests (Holt, et al., 1994) were done (Fig. 9-13, Fig.15), which were:

a) IMViC test-Indole, MR,VP and Citrate test
b) Carbohydrate test-included Dextrose, Sucrose, Maltose, Mannitol, Mannose, Sorbitol, Fructose, Glycerol, Lactose, Arabinose and Starch.
c) Nitrate reduction test
d) Starch hydrolysis test (Fig. 10)
e) Casein utilization test
f) Temperature tolerance test (50°C) (Fig. 12)

g) 5% NaCl Test

h) 7% NaCl Test

i) Acetate utilization test

j) Urease test

k) Motility test (Fig.13)

l) Catalase test (Fig. 11)

Further tests were performed for yeast identification (figure 15), which were:

- KNO_3 Test
- Germ tube test

Fig9: showing biochemical assays for the presence of different microbial strains at different time intervals of Aerobic and anaerobic liver samples. Tests include (a) Indole test, (b) MR test, (c) Citrate test, (d) Acetate utilization test, (e),(f) and (g) Carbohydrate test-Glucose, Sucrose, Maltose (h) VP test

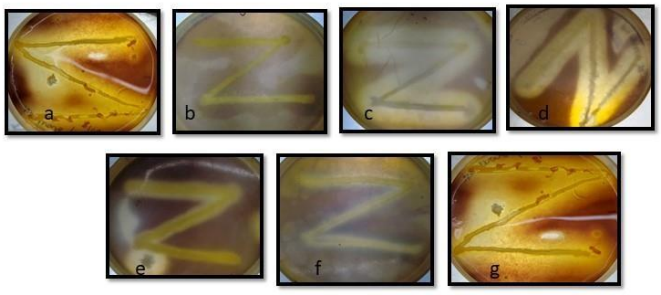

Fig:10. Starch Hydrolysis Test of aerobic and anaerobic liver samples in soil and water (a) Short Rods, Gram –ve (b) Streptobacillus with endospores, Gram +ve (c) Cocii, Gram +ve (Yellow pigmentation) (d) Rods, Gram +ve (e) Short Rods, Gram +ve (f) Yeast cells (Produces brownish pigment) (g) Very Short Rods, Gram -ve

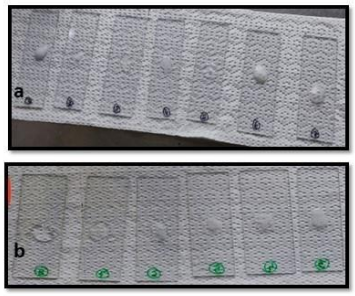

Fig.11: Catalase Test
(a) Aerobic samples
(b) Anaerobic samples

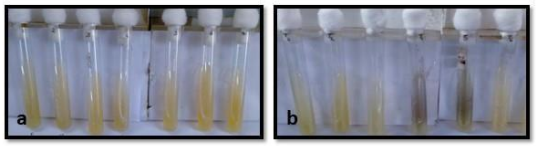

Fig.12: Temperature tolerance Test
(a) Aerobic samples and (b) Anaerobic samples

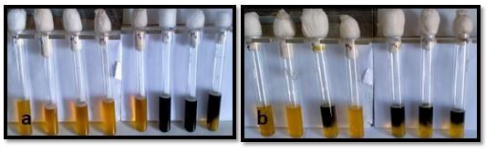

Fig.13: Motility Test
(a) Aerobic samples and (b) Anaerobic samples

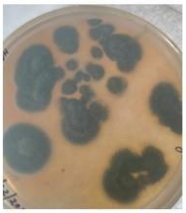

Fig.14: Fungi

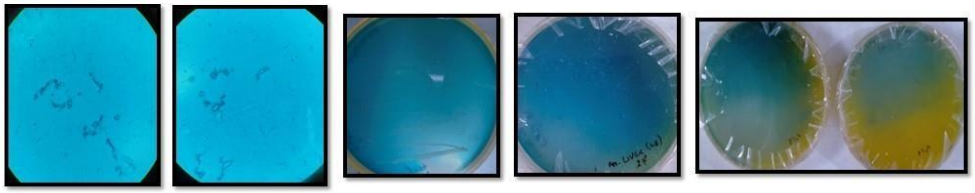

Fig.15: Some specific tests for yeast identification
(a) Germ tube test, (b) and (c) KNO_3 Test at 27°C and 4°C

Species identification was done by comparing biochemical results over Bergy's software (Holt, et al., 1994). 16sRNA analysis was conducted on particular strains. HiPurA Bacterial DNA purification 24 spin column kit (MB505-250PR, HiMedia, India) was used to isolate the DNA, and was analysed using 1% agarose gel electrophoresis. Using primers F27 (5'AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' GGTTACCTTGTT ACGACTT 3'), the bacterial-specific 16s rRNA gene (1500 bp) was amplified by PCR (Clarridge, III, 2004). 1.25 µL of each primer (10 µM), 1 µL of the DNA template (50–100 ng), and 12.5 µL of the EmeraldAmp GT PCR Master Mix, 2x (Takara Bio USA) (forward and reverse), and the PCR reaction was performed in a 25 µL volume using 9 µL of free-nuclease water.

PCR amplification was carried out using an Applied Biosystems Veriti Thermal Cycler, and the parameters used were as follows: Denaturation for five minutes at 94°C, then thirty seconds at 94°C, twenty-five seconds at 55°C, one minute at 72°C, and one minute at 72°C for a final cycle. Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) under the following conditions: 28 cycles of 96°C for 1 min, 50°C for 5 s, and 60°C for 4 min after denaturation for 1 min was used. Thermo Fisher Scientific's sodium acetate ethanol technique was used to purify cycle sequenced amplicons, and a 3500xL Genetic analyser (Applied Biosystems, USA) was used for sequencing. The National Centre for Biotechnology Information (NCBI) database was used to compare the closest culture sequence to edit sequencing files (.ab1) using CHROMASLITE (version 1.5). The results were then analysed using Basic Local Alignment Search Tool (BLAST) to find areas of local similarity between the sequences (Altschul et al., 1990).

RESULT:

I. Culture-dependent characterization of the thanatomicrobiota:

The culture-dependent characterization of the thanatomicrobiota in liver and kidney tissue of goat model was done in soil and water samples at different time and temperature conditions. The characterization involves the isolation and purification of the microbiota obtained on the decomposed tissue across time and temperature variations. The isolated bacterial and fungal strains were gram stained for morphological differentiation followed by biochemical tests. The test results were then applied on the Bergey's identification application. The results obtained showed percentage similarity to genus and species of the bacterial cultures (Table 1-7). The pure cultures were separated for further analysis.

Gram Staining Results

Temp	Time (Hours)	S.No.	Aerobic liver culture	Anaerobic liver culture	Aerobic kidney culture	Anaerobic kidney culture
37ºC	3	Soil	Gram-negative shorts rods	Gram-negative Short rods	Short rods Gramnegative, Grampositive rods	Short rods Gramnegative
		Water	Gram-positive Cocci, Gram-negative short rods	Gram negative short rods, Gram positive Cocci	Short rods Gram negative	Gram positive rods, short rods Gramnegative
	24	Soil	Gram-positive rods,Gram-negative shorts rods	Gram-negative shorts rods	Gram-negative shorts rods, Gram positive Cocci, Gram positive rods with endospores	Gram negative Short rods
		Water	Gram-positive rods,Gram-negative shorts rods	Gram-negative shorts rods	Gram-negative shorts rods	Gram-positive rods,Gramnegative shorts rods
	48	Soil	Gram-negative short rods	Gram positive Cocci	Gram-negative shorts rods	Gram-negative shorts rods
		Water	Gram-negative short rods, Gram positive Cocci	Gram-negative short rods	Gram-negative short rods, Gram positive Cocci	Gram positive Cocci, Gramnegative short rods
	72	Soil	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods
		Water	Gram-negative short rods	Gram positive Bacillus	Gram positive Bacillus	Gram positive rods, Gram-negative short rods
27ºC	24	Soil	Gram positive Cocci	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods
		Water	Gram positive Cocci (yellow and white pigmented)	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods, Gram positive rods
	48	Soil	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods and Gram positive Cocci	Gram-negative short rods and Gram positive Cocci
		Water	Gram-negative short rods	Yeast Cells	Gram-negative short rods and Gram positive Cocci	Gram-negative short rods and Gram positive rods
	72	Soil	Gram-negative short rods and Gram positive Cocci	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods
		Water	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods and Gram positive Cocci	Gram positive Cocci, Gram positive rods

4ºC	24	Soil	Gram-negative short rods	Gram-negative short rods and yeast	Gram positive Cocci	Gram positive Cocci
		Water	Gram-negative short rods	Gram positive Cocci, Gramnegative short rods	Gram-negative short rods	Gram-negative short rods
	48	Soil	Gram-negative short rods	Gram-negative short rods and yeast	Gram-negative rods	Gram-negative short rods and yeast
		Water	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods
	72	Soil	Gram positive Cocci	Gram-negative short rods	Gram-negative short rods	Gram-negative short rods
		Water	Gram-negative short rods	Gram-negative short rods, Yeast Cells	Gram-negative short rods	Gram-negative short rods

Table 1: Gram staining results of liver and kidney culture samples at different temperature and time interval in soil and water.

Aerobic liver culture					**Anaerobic liverculture**				
Strain 1	37ºC	0hour	Soil	Gram-negative short rods	Strain 1	37ºC	0hour	Water	Gram positive, Cocii(White Pigmented)
Strain 2	37ºC	0hour	Water	Gram Positive, Streptobacillus with endospores	Strain 2	37ºC	0hour	Water	Short Rods with thick edges, Gram positive
Strain 3	27ºC	0hour	Water	Gram positive, Cocci (Yellow pigmentation)	Strain 3	27ºC	72hour	Water	Gram positive, rods
Strain 4	27ºC	0hour	Water	Gram positive, Cocci (Yellow pigmentation)	Strain 4	27ºC	0hour	Water	Gram negative, Short Rods (Yellow Pigmented)
Strain 5	37ºC	24hour	Water	Gram negative, Short Rods	Strain 5	37ºC	48hour	Soil	Gram negative, Short Rods
Strain 6	37ºC	48hour	Soil	Gram negative, Short Rods	Strain 6	37ºC	0hour	Soil	Gram negative, very short Rods
Strain 7	37ºC	72hour	Water	Gram negative, very short Rods	Strain 7	27ºC	48hour	Water	Yeast Cells
				Gram negative, Short Rods	Strain 8	27ºC	72hour	Water	Yeast Cells

Table 2: Selected strains of liver sample at different temperature and time interval in soil and water

Aerobic Kidney					**Anaerobic Kidney**				
Strain 1	37ºC	3hour	Soil	Gram positive rods with endospores	Strain 1	37ºC	3hour	Soil	Gram positive rods with endospores
Strain 2	27ºC	3hour	Water	Gram negative, short Rods (Greyish pigmented)	Strain 2	37ºC	3hour	Water	Gram positive rods with endospores
Strain 3	27ºC	0hour	Water	Gram positive Cocci	Strain 3	27ºC	3hour	Water	Gram positive Cocci (Orange pigmented)
Strain 4	37ºC	24hour	Water	Gram negative, short Rods	Strain 4	37ºC	24hour	Soil	Gram negative, short Rods
Strain 5	27ºC	24hour	Water	Gram negative, short Rods (Yellow pigmented)	Strain 5	37ºC	24hour	Water	Gram negative, short Rods
Strain 6	37ºC	48hour	Soil	Gram negative, short Rods	Strain 6	37ºC	48hour	Water	Gram negative, short Rods
Strain 7	37ºC	48hour	Water	Gram negative, short rods	Strain 7	27ºC	48hour	Water	Gram negative, very short rods
Strain 8	37ºC	72hour	Soil	Gram negative, short rods					

Table 3: Selected strains of kidney's culture at different temperature and time interval in soil and water

Biochemical tests:

	Aerobic Liverculture							Anaerobic Liverculture							
TEST	L1	L2	L3	L4	L5	L6	L7	L1	L2	L3	L4	L5	L6	L7 (Yeast Cells)	L8 (Yeast Cells)
Indole	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
MR	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve
VP	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Citrate	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Dextrose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Mannitol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Maltose	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
Sorbitol	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Mannose	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve

Fructose	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Arabinose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
Starch	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
Glycerol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Nitrate reduction	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
Urease	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Starch hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7% NaCl	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5% NaCl	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Acetate utilization	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Temperature tolerance	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
(50ºC)															
Casein Hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Motility Test	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve

Table 4: Biochemical tests result for Liver culture sample at different temperature and time interval in soil and water

Yeast identification tests for sample L7 and L8:

Germ Tube Test	+ve
KNO3 Test (27ºC)	+ve
KNO3 Test (4ºC)	+ve

Table 5: Yeast identification tests for sample L7 and L8

	Aerobic kidney culture								Anaerobic kidney culture						
TEST	L1	L2	L3	L4	L5	L6	L7	L8	L1	L2	L3	L4	L5	L6	L7
Indole	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve
MR	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
VP	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Citrate	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Dextrose	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
Sucrose	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve
Mannitol	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
Maltose	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve

Sorbitol	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
Mannose	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Fructose	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Lactose	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
Arabinose	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
Starch	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Glycerol	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Nitrate reduction	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Urease	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Starch hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7% NaCl	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
5% NaCl	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Acetate utilization	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Temperature tolerance (50ºC)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Casein Hydrolysis	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Motility Test	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve

Table 6: Biochemical tests result for Kidney sample for different temperature at different time interval under soil and water

Further, the bacterial cultures were identified by comparing the biochemical results on Bergey's programme

Culture	Strain Number	Possible microorganisms	Percentage
Aerobic liver culture	Strain 1	*Enterobacter asburiae*	85.1%
	Strain 2	*Bacillus mycoides*	86.4%
	Strain 3	*Micrococcus lylae*	98.1%
	Strain 4	*Nesterenkoniamassiliensis*	86.3%
	Strain 5	*Cedecea species 5*	87.6%
	Strain 6	*Cedecea species 5*	92.8%
	Strain 7	*Mangrovibacterplantisponsor*	89.2%
Anaerobic liver culture	Strain 1	*Staphylococcus scluri*	85.3%
	Strain 2	*Bacillus cereus*	87%
	Strain 3	*Bacillus coagulans*	91.9%
	Strain 4	*Pseudomonas (Flavimonas) oryzihabitans*	80.4%

	Strain 5	*Burkholderiamultivorans/B. cepacian genomovar II/VI*	90.8%
	Strain 6	*Pseudomonas oleovorans (P. pseudoaligenes)*	89.2%
	Strain 7	*Candida sp*. (Yeast Cells)	
	Strain 8	*Candida sp*. (Yeast Cells)	
Aerobic kidney culture	*Strain 1*	*Bacillus licheniformis*	*86%*
	Strain 2	*Pseudomonas aeruginosa*	*85%*
	Strain 3	*Staphylococcus intermedius*	*86%*
	Strain 4	*Proteus mirabilis*	*86%*
	Strain 5	*Advenellakashmirensisn*	*87%*
	Strain 6	*Cedaecealapagaei*	*84%*
	Strain 7	*Enterobacter carcerogenous*	*85%*
	Strain 8	*Cedaecealapagaei*	*84%*
Anaerobic kidney culture	*Strain 1*	*Bacillus Subtilis*	*85%*
	Strain 2	*Bacillus megatarium*	*83%*
	Strain 3	*Staphylococcus aureus*	*84%*
	Strain 4	*Losdaleaquercina*	*83%*
	Strain 5	*Erwinia amylovora*	*82%*
	Strain 6	*Serratia entomophila*	*92%*
	Strain 7	*Enterobacter cloacae*	*85%*

Table 7: Identification of microbial cultures using Bergey's programme

The result showed the presence of fungi at 27°C (72hr.) (Fig.14) which found to be *Gliocladiumveruculasum* and *Tricodermaveruculasum*, which is very common in vegetarians (Sidrim, et al., 2010)**.** Growth of two different types of yeast strains (Fig.7) and fungi, *Gliocladium and Tricodermaveruculasum* (Fig.14) were found which were the unique discoveries of this study. These microorganisms were developed at the later stages of death (more than 48hr.) and at low temperatures (4°C and 27°C, predominately at 27°C). The Development of maggots was also noticed after 48hours of incubation (Fig.6).

In the present study, *Bacillus spp.* and *Staphyloccocus spp.* can be considered as biomarker of liver tissue depicting a PMI of 3-24hrs., in aerobic condition. After 48hr. and 72hr. post-mortem interval (PMI), the microbiota succession changed to Proteobacteria, showing presence and growth of mainly gram-negative bacilli strains. The identification of different bacteria and yeast show similar results as earlier studies by Tuomisto, et al., 2013 & Williams et al., 2010 where the study showed existence of certain class of bacteria which developed with organ's decomposition and PMI elapsed.

II. Culture-Independent description of the thanatomicrobiota in Liver and Kidney

The culture-independent characterization of the thanatomicrobiota in liver and kidney tissue of goat model was done in soil and water samples at different time and temperature conditions. Genetic material from the sample of interest can be directly extracted utilizing cultureindependent procedures. It is now possible to locate and describe a wide variety of microbial species that were previously indefinable or impossible to identify, and this allows for the highthroughput profiling

of the microbial community through genome sequencing (Gill et al., 2006; Schloissnig, et al., 2013; Adserias-Garriga, et al., 2017a; Almeida et al., 2019, Sunagawa, et al., 2013). In the current study, a genomic segment which is retained in a taxon but variable among taxa is augmented. The typical marker gene is the 16S rRNA gene for bacterial cultures and the internal transcribed spacer (ITS) for yeast and other filamentous fungi (Zhou and Bian, 2018) and evaluation of 16S rRNA gene sequencing for strain and species-level microbiome analysis (Johnson, et al., 2019). The most popular genetic marker for bacteria that is used to determine the types of microorganisms that are present in a community is the 16S rRNA gene. It is a single phylogenetic marker that can provide a more comprehensive coverage but cannot discriminate between different species.
A brief section was amplified following the 16S rRNA gene analysis and subsequently sequenced. The identification of the microbiota was then achieved by searching homologous gene sequences in the data bank using the NCBI-BLAST programme. The National Centre for Biotechnology Information (NCBI) closest cultural sequence in the database was used to edit sequencing files (.ab1) using CHROMASLITE (version 1.5). The data was then analysed using the Basic Local Alignment Search Tool (BLAST) to find areas of local similarity between the sequences (Altschul et al., 1990)**.** The results obtained showed presence of variety of microorganisms viz, *Pseeudomonas aeruginosa (99%), Yarrowia lipolytica (97.2%), Dipodascaceae sp. (99%), Serratia marcescens (99.07%), Serratia nematodiphila (95.75%), Enterobacter sp. (95.63%).*

DISCUSSION:

The human microbiome project (Yuan, et al., 2022, Hamady, et al., 2009, Turnbaugh, et al., 2007) proved that the total number of microbial cells in and on the surface of the human body is ten times higher than that of human cells. The presence of these microbes varies depending on the area of the body. Particular body parts are colonized by particular microbial assemblages and microbes. The variables that influence the microbial assemblage are temperature, time, host health, and diseases. Despite being dynamic, these changes are frequently very predictable. The molecular interactions between the microbiome and the extrinsic and intrinsic factors are fascinating, but what's even more exciting is the possibility that the microbiome could operate as a microbial marker for these aspects. The findings by Vass (1992) to determine time since death of human cadavers using soil solution was comparable with the outcomes of this study (Vass, 1992; Megyesi. et al., 2005)**.**

In light of veterinarian postmortem testing in a model species, the present study aims to provide a systemic investigation of the thanatomicrobiome in environmental settings. The PMI microbiota was studied using the goat liver and kidney, which were derived from the model animal *Capra aegagrus hircus*. PMI upto 72 hrs was focused specially being diagnostically meaningful. Instead of sampling the same organs under soil and water in both aerobic and anaerobic conditions, the design of the study allowed for the collection of samples from different animals at various times and temperatures.

CONCLUSION:

In conclusion, the intricate interplay between soil and water dynamics significantly influences the succession of thanatomicrobiome communities, thereby impacting the estimation of postmortem interval (PMI). Through comprehensive research and analysis, it becomes evident that soil and water conditions play pivotal roles in shaping microbial colonization patterns on decaying remains. Understanding these ecological processes is crucial for enhancing the accuracy of PMI estimation methods, which hold profound implications for forensic investigations and criminal justice proceedings. Moving forward, continued interdisciplinary research and technological advancements will further elucidate the complexities of thanatomicrobiome succession, ultimately refining our ability to ascertain the timing of death with greater precision and reliability.

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