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Effect Of Tissue Preservation Chemicals On Decomposition In Different Soil Types

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ABSTRACT

Forensic taphonomy involves varied range of factors- decomposition processes, chemical and biological interactions with cadavers, and the chronology of events preceding death, which contribute to reconstruction of events and estimation of post mortem interval (PMI). This research aimed to assess the quality of DNA extracted from embalmed tissues of *Sus domesticus* buried in different soils and the soil enzyme quality after 1 year. *Sus domesticus* limbs (n=36) were randomly allocated into three groups (A-C) of, three treatment samples and one control sample. Before burial, each sample was treated in either 10% formaldehyde, absolute methanol, or 50% Pine oil for 24hr except the control samples. The limbs were buried in 36 simulated graves containing clay, sandy, or loamy soil types. DNA quality was assessed by extracting and quantifying DNA from the individual samples. The rate of decomposition was evaluated using modified qualitative decomposition analysis. Biochemical enzyme assays were conducted on the soil from each grave. All the buried limbs experienced weight loss. The formaldehyde preserved samples exhibited significant increase ($p \leq 0.01$) in soil urease levels. The control grave soils showed significantly higher ($p \leq 0.01$) alkaline phosphatase, dehydrogenase, and calcium carbonate values compared to the experimental grave soils. The experimental samples demonstrated a significant decrease ($p \leq 0.01$) in DNA concentration and purity compared to the control groups. The results showed that the embalming treatment altered the relationship between organic matter decomposition and soil biochemical properties. When conducting investigations, it's important to consider various factors in determining the PMI. These factors include the characteristics of the grave soil, the soil biochemical properties, plus any pre-mortem exposure to embalming chemicals. Considering these factors, investigators can effectively estimate PMI, which is crucial in forensic analysis and establishing the timeline of events surrounding a crime.

Keywords: Forensic Taphonomy, Post-mortem Interval (PMI), Embalming, Decomposition, Grave Soil, Biochemical Properties.

INTRODUCTION

Decomposition is a natural phenomenon that holds significant importance in the energy cycling process of ecosystems. Through human and animal studies, researchers have gained extensive knowledge regarding the disintegration of soft tissues and alteration of hard tissues, shedding light on the variability and progression of decomposition. These studies have uncovered numerous factors that influence the nature and timing of this process. Temperature and the specific environment have traditionally been recognized as crucial considerations. However, it has become evident that various additional factors exert influence. Soil conditions, moisture levels, body composition, overall body condition, the presence of clothing or enclosures, funerary treatments, and various other elements have all been found to impact the decomposition process. Accurate assessment of the post-mortem interval (PMI) and understanding post-mortem events associated with criminal activities require a comprehensive understanding of these factors and their interplay (Ubelaker, 2018). There is a growing demand to expand the current understanding of forensic taphonomy, particularly regarding the dynamics of tissue decomposition within terrestrial ecosystems and tropical climatic conditions found in Sub-Saharan Africa and other regions across the globe. This need arises due to the alarming rise in incidents of violent murders, genocide, ethnic and inter-tribal mass killings, where the true intentions behind these crimes are often concealed through the use of

embalming techniques and burial in shallow clandestine mass graves.

In order to accurately estimate the PMI and identify potential suspects, research efforts must be directed towards filling the knowledge gaps in this field. The unique environmental and climatic conditions of these regions necessitate a specific focus on understanding the decomposition process and associated factors within this context. By conducting thorough research, investigators can enhance their ability to accurately determine PMI, unravel the circumstances surrounding these heinous crimes, and bring the perpetrators to justice.

The influence of soil particle size on the decomposition process is widely recognized. Coarse-textured soils, characterized by larger particles, have been found to impede or significantly reduce decomposition due to limited gas diffusion through the soil matrix. In such soil conditions, the decomposition process can be either completely blocked or reduced to minimal levels (Tibbett et al. 2004). Tissue embalming or preservation refers to the practice of injecting chemical substances into the body of a deceased individual following death with the aim of preventing or slowing down the natural process of decay. By introducing these chemicals, the decomposition of tissues is inhibited, allowing for the preservation of the body for an extended period of time. This technique is commonly used in mortuary practices, funeral services, and forensic investigations to temporarily maintain the physical appearance of the deceased and facilitate various post-mortem procedures (Balta et al. 2019). After death, cellular decay sets in rapidly, leading to the quick deterioration of cells and causing oxidative and hydrolytic damage to DNA. To counteract this process, embalming techniques are employed to preserve tissues. The preservation is achieved by utilizing fixatives that trigger molecular cross-linking within the tissues, thereby slowing down the decay process. The cross-linking induced by the fixatives helps stabilize cellular structures and minimizes the extent of DNA damage. In this way, embalming serves as a means to preserve tissues and maintain the integrity of DNA molecules, allowing for potential forensic analysis or other investigative procedures that rely on DNA evidence (Gielda and Rigg, 2017). The primary objective of embalming is to ensure the preservation of both hard and soft bodily tissues by preventing putrefaction, disruptions, and infestation by insects and maggots. Various chemical substances are utilized in the embalming solution to achieve these goals. These substances include formaldehyde, glutaraldehyde, phenol, glycerin, bronopol, ethanol, glycol, as well as natural extracts like pine oil, cedar wood oil, natron, myrrh, vanillin, and others. The combination of these chemicals and natural extracts helps in the process of tissue preservation by inhibiting decomposition, reducing microbial growth, and providing a barrier against insect activity (Balta et al. 2019).

In the field of forensic entomology and taphonomy, a majority of studies on decomposition have relied on non-human cadavers. In the 1980s, a recommendation was made to use domestic pig cadavers as analogues for human cadavers in forensic entomology. As a result, pigs have become the most commonly utilized model cadavers in forensic sciences. The use of pig cadavers has significantly contributed to our understanding of decomposition processes in large vertebrate cadavers within various environments, seasons, and under different ante- or postmortem conditions. Pig cadavers have played a crucial role in demonstrating the feasibility and effectiveness of several new or established forensic techniques. They have helped researchers assess and refine methods for estimating the post-mortem interval, understanding decomposition patterns, and studying the effects of various factors on the decay process. The establishment of outdoor human taphonomy facilities has further facilitated experimental comparisons between pig and human cadavers. These facilities have provided the opportunity to directly observe and compare the decomposition processes and associated changes in both pig and human remains under controlled conditions. This has greatly enhanced our knowledge and allowed for more accurate interpretations in forensic investigations (Matuszewski et al. 2020). Burials are a frequent way for disposing of human remains in homicide cases, and many of them are secret or close to the surface. The decomposing remains will have an impact on the surrounding soil, similar to surface decomposition, though with subterranean burial, the impact on the body from the soil environment is larger. Burial is one of the strategies that can be utilized to keep the body hidden from view

(Enwere, 2008). The depth of this type of shallow clandestine cemetery is usually between 0.3 and 0.7 m, as opposed to 1.2 to 1.4 m in a legitimate graveyard (Prangnell and McGowan, 2009).

Furthermore, in criminal cases where there is a lack of reliable witnesses, positive DNA identification, or fingerprints, forensic soil analysis can be utilized by investigators to help narrow down their investigation to a particular individual or geographic area (Singh et al. 2023). The structure of any decomposing organic matter influences and causes changes in soil biochemical characteristics. As a result, soil qualities and textures should be addressed in forensic sciences when evaluating decomposition and determining postmortem intervals in crime scene investigations (Naseby and Lynch, 1997). During the process of carrion decomposition, it is anticipated that the chemistry of the surrounding soil will undergo changes due to the release of nutrients from the decaying carcass. This alteration in nutrient concentration within the soil can be assessed through soil chemical analysis, including parameters such as pH, electrical conductivity, moisture content, extractable soil phosphorus, total carbon, total nitrogen, ammonium, nitrate, sulfur, and iron. Forensic soil scientists can utilize this analysis to make a more accurate estimation of the time of death by comparing soil samples taken from the area surrounding the carrion with control soil samples that were not exposed to carrion (Singh et al. 2023). Therefore, in this study, we conducted measurements of urease, alkaline phosphatase, dehydrogenase, and $CaCO_3$ levels in the soil of the graves. Additionally, the molecular identification of the decomposing samples through DNA

Table 1: Sample grouping, weight and preservation chemicals

Groups	No of laps	Mean weight(kg)	Preservation Chemical
A	12	A_1- 1.4	10 % Formaldehyde
		A_2 - 1.4	Methanol
		A_3 - 1.2	50 % pine Oil
		Control - 1.0	
B	12	B_1 - 1.2	10 % Formaldehyde
		B_2 - 1.2	Methanol
		B_3 - 0.8	50 % Pine Oil
		Control - 0.9	
C	12	C_1 - 0.9	10 % Formaldehyde
		C_2 - 0.8	Methanol
		C_3 - 0.7	50 % Pine Oil
		Control - 0.8	

Materials used for the study

The experiment involved the use of nine male piglets as the subjects. The embalming process utilized three different chemicals: 10% formaldehyde, methanol, and 50% pine oil. Other equipment and materials utilized during the experiment included tulles nets, a shovel, a digger, a wheelbarrow, a weighing scale, a measuring tape, soil samples of sandy, clay, and loam soils, hand gloves, plain bottles, plastic containers, isopropanol, sodium chloride, and ethidium bromide.

Burial of Samples

To ensure proper separation between the sample soil and the surrounding host soil within the graves, small porous tulles nets were placed inside the graves. The nets served as a barrier, preventing mixing between the two types of soil. The soil samples used in the study were then filled into the graves, with Figure 1 illustrating the placement of all samples in a horizontal position at the center of the graves. The remaining space in the graves was subsequently filled with the sample soil. This approach aimed to simulate the actual burial soil conditions accurately and minimize any potential interaction between the host soil and the samples, thus maintaining the integrity of the experimental setup.

Figure 1: Burial process of the pig limbs (A) the laying of the small porous tullen before filling with sand (B) Shows filled grave with burial soil and the pig extremity placed horizontally (C) the completion of the burial process and covering the grave with the burial soil.

Sample Exhumation and Evaluation of Decomposition

After precisely 12 months, the buried extremities in the graves were excavated (refer to Figure 2). These decomposed extremities were weighed to quantify the extent of mass loss that had occurred. Furthermore, detailed photographs were taken to document the visible changes and decay that had taken place.

A qualitative assessment of decomposition was conducted based on visual parameters, which were derived from previous research (Tumer et al. 2013). The parameters examined included skin corruption, tissue loss, hoove and bone disarticulation (separation), formation of adipocere (waxy substance resulting from decomposition), and the presence of decomposing hair. By analyzing and recording these visual parameters, a comprehensive understanding of the decomposition process was obtained. This information contributed to the assessment of the extent and progression of decomposition over the 12-month period.

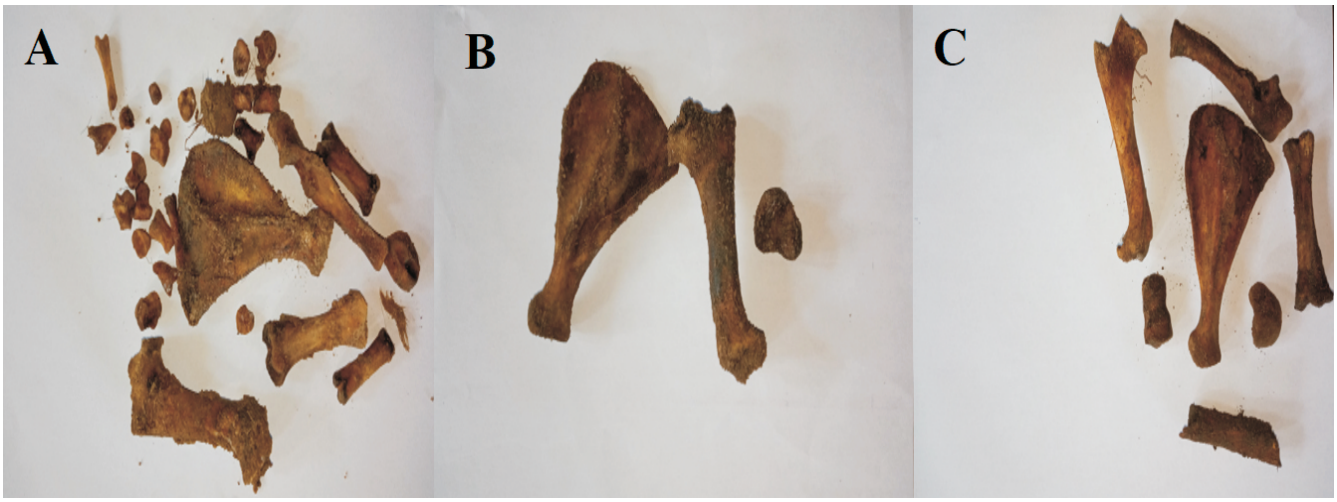

Figure 2: Cross-section of exhumed putrefied bones of buried pig limbs after 12 months.

Evaluation of decomposition

The qualitative assessment of decomposition status was conducted by evaluating visual parameters based on modifications from the previous work referenced as (Tumer et al. 2013). The observed parameters included the assessment of skin corruption, loss of soft tissue, disarticulation of bones and hooves, as well as the formation of adipocere. These parameters were comprehensively evaluated to gain insights into the state of decomposition and the changes that occurred during the study period.

Bone decontamination and deoxyribonucleic acid (DNA) extraction.

After the bones were exhumed, they underwent a series of steps for DNA extraction. The initial step involved cleaning the bones to remove any adherent tissues, sand particles, and dirt from the surface. This cleaning process was carried out using deionized water followed by 95% ethanol, using sterile cotton wool. Next, the cleaned bone samples were air dried overnight at room temperature in a sterilized environment. Once dried, the collected bone samples were cut into smaller pieces to obtain approximately 0.6-1g of bone powder. Each sample was prepared individually, utilizing sterile and disposable tools, in an isolated area to minimize the risk of contamination. The salting out DNA extraction protocol modified from Medrano (Medrano et al. 1990) was adopted for the extraction of pure DNA from about 0.1g of bone powder from the samples. The bone powders were digested in proteinase K at 55°C for 48hours following other procedures.

Spectrophotometry

To assess the quality and quantity of DNA obtained from the samples, spectrophotometric analysis was performed using a Nanodrop 1000 spectrophotometer (specifically, the 6305 JENWAY spectrometer model). This analysis involved measuring the absorption ratios of A260/230 and A260/280.

DNA Amplification and Gel Electrophoresis

The COI gene was amplified using primers LCO1490
(5' GGTCAACAAAATCATAAAGATATTGG3') and HCO2198
(5' TAAACTTCAGGGTGACCAAAAAATCA 3') from Hebert et al. (2003). For the Polymerase Chain Reaction (PCR) amplification, a 25 µl reaction mix was prepared. The components of the reaction mix included 6.5 µl of nuclease-free water, 12.5 µl of DNA master mix, 0.5 µl of each forward and reverse primer, and 5 µl of DNA template. The PCR amplification program was optimized and designed for efficient DNA amplification. It consisted of an initial denaturation step at 94 ºC for 3min, followed by five cycles of denaturation at 94 ºC for 40 sec, annealing at 52 ºC for 30 sec, and extension at 72 ºC for 45 sec. This was allowed to run for 30 cycles and finally, a final extension step was performed at 72 ºC for 7 mins. After PCR amplification, the products were visualized using a 2.0% agarose gel containing ethidium bromide. The gels were post-stained and then viewed using the Molecular Imager® Gel DocTM XR System (Bio-Rad, USA) (Lee et al. 2012).

Soil Analyses

The dehydrogenase activity of the soil samples, which is an indicator of the carbon cycle, was determined using the protocols described in reference (Thalmann, 1968). The alkaline phosphatase activity, which reflects the phosphorus cycle, was analyzed following the protocols outlined by (Tabatabai and Bremner, 1969). To assess the urease activity of the soil samples, which represents the nitrogen cycle, the methods described by Sinsabaugh and Linkins (1988) and Kandeler et al. (1996) were used respectively. The percentage of carbonate in the soil samples was determined using the volumetric calcimeter method recommended by the Food and Agricultural Organizations. By analyzing these various soil parameters and activities, a comprehensive understanding of the soil's biochemical properties and nutrient cycling can be obtained, providing valuable insights into the decomposition process and post-mortem interval estimation.

Statistical Analysis

The numeric data obtained from the study were analyzed using IBM SPSS Version 26 software (IBM SPSS Inc., USA). Pearson correlation analysis was performed to compare the mean values and determine any significant differences. The results were expressed as Mean ± SE (standard error). Statistical significance was set at $P \leq 0.01$. Graphs illustrating the data were created using Microsoft Excel Version 16.

RESULTS

Spectrophotometry

Table 2 shows the descriptive relationship between concentration and purity across the 3 soil burial groups. In the A group, the blank control had the highest concentration Mean ± SEM (98.50 ± 1.15 ng/uL) value while the A2 sample had the least (5.80 ± 1.15 ng/uL). The DNA extracted from the blank control group also had the highest purity Mean ± SEM (1.86 ± 0.12 ng/uL) while that of the A control was the least pure (1.32 ± 0.012 ng/uL).

Table 2: Descriptive Statistics of the Extracted DNA Purity and Concentration

	Range	Minimum	Maximum		Variance
A1 Purity	0.22	1.46	1.68		0.015
A1 Conc. (ng/ul)	4.00	11.60	15.60	13.60±1.15	4.000
A2 Purity	0.04	1.31	1.35	1.33±0.12	0.000
A2 Conc. (ng/ul)	4.00	3.80	7.80	5.80±1.15	4.000
A3 Purity	0.04	1.45	1.49	1.47±0.016	0.000
A3 Conc. (ng/ul)	4.00	26.20	30.20	28.20±1.155	4.000
A Ctrl Purity	0.04	1.30	1.34	1.32±0.012	0.000
A Ctrl Conc. (ng/ul)	4.00	17.50	21.50	19.50±1.15	4.000
A Blank Ctrl Purity	0.40	1.66	2.06	1.86±0.12	0.040
A Blank Ctrl Conc. (ng/ul)	4.00	96.50	100.50	98.50±1.15	4.000
B1 Purity	0.04	1.32	1.36	1.34±0.012	0.000
B1 Conc. (ng/ul)	3.00	80.30	83.30	81.63±0.88	2.333
B2 Purity	0.04	1.42	1.46	1.44±0.12	0.000
B2 Conc. (ng/ul)	4.00	10.60	14.60	12.60±1.15	4.000
B3 Purity	0.04	1.30	1.34	1.32±0.012	0.000
B3 Conc. (ng/ul)	4.00	29.30	33.30	31.30±1.15	4.000
B Ctrl Purity	0.04	1.26	1.30	1.28±0.012	0.000
B Ctrl Conc. (ng/ul)	4.00	10.00	14.00	12.00±1.15	4.000
B Blank Ctrl Purity	0.40	1.68	2.08	1.88±0.12	0.040
B Blank Ctrl Conc. (ng/ul)	4.00	104.50	108.50	106.50±1.15	4.000
C1 Purity	0.04	1.23	1.27	1.25±0.012	0.000
C1 Conc. (ng/ul)	4.00	39.00	43.00	41.00±1.15	4.000
C2 Purity	0.40	1.51	1.91	1.71±0.11	0.040
C2 Conc. (ng/ul)	4.00	1.40	5.40	3.40±1.15	4.000
C3 Purity	0.04	1.31	1.35	1.33±0.012	0.000
C3 Conc. (ng/ul)	3.00	4.50	7.50	6.16±0.88	2.333
Cs Ctrl Purity	0.40	1.27	1.67	1.47±0.12	0.040
C Ctrl Conc. (ng/ul)	4.00	8.90	12.90	10.90±1.15	4.000
C Blank Ctrl Purity	0.02	1.95	1.97	1.96±0.007	0.000
C Blank Ctrl Conc. (ng/ul)	4.00	108.90	112.90	110.90±1.15	4.000

Legend

A1 = Clay Soil/Formaldehyde Treatment Group
A2= Clay Soil/Methanol Treatment Group
A3 = Clay Soil/Pine Oil Treatment Group
B1 = Sandy Soil/Formaldehyde Treatment Group
B2= Sandy Soil/Methanol Treatment Group
B3= Sandy Soil/Pine Oil Treatment Group
C1= Loamy Soil/Formaldehyde Treatment Group
C2= Loamy Soil/Methanol Treatment Group
C3= Loamy Soil/Pine Oil Treatment Group

Absorbance ratios for A260/A280 were determined for 1µl of each sample across the 3 groups using a Nanodrop 1000 spectrophotometer (6305 JENWAY). Table 3 shows the Pearson correlation analysis of the spectrophotometry values. The level of DNA Concentration in the A1 sample correlated with concentration level in A2, A3, Positive control, blank control, purity levels in

sample A3 and positive control ($p \leq 0.01$). Also, A2 concentration levels correlated with concentration values in A3, positive control, blank control DNA purity level in A3 and positive control ($p \leq 0.01$). The levels of DNA purity is correlated with concentration levels in A2, A3, positive control, blank control ($p \leq 0.01$). Furthermore, In the B group, the blank control sample has the highest concentration Mean ± SEM (106.50 ± 1.15 ng/uL) and purity of DNA (1.88 ± 0.12 ng/ul) while the B control sample had the least DNA concentration

Table 3: Pearson Correlation Analysis Output of Spectrophotometry Values-GROUP A

	A1 Purity	A1 Conc. (ng/ul)	A2 Purity	A2 Conc. (ng/ul)	A3 Purity	A3 Conc. (ng/ul)	A Ctrl Purity	A Ctrl Conc. (ng/ul)	A Blank Ctrl Purity	A Blank Ctrl Conc. (ng/ul)
A1 Purity	1.000	0.904	0.822	-0.904	0.904	-0.904	0.904	-0.904	-0.082	0.904
A1 Conc. (ng/ul)	0.904	0.281 1.000	0.386 0.500	0.281 - 1.000**	0.281 1.000**	0.281 - 1.000**	0.281 1.000**	0.281 - 1.000**	0.948 -0.500	0.281 1.000**
A2 Purity	0.281 0.822	0.500	0.667 1.000	0.000 -0.500	0.000 0.500	0.000 -5.00	0.000 0.500	0.000 -0.500	0.667 0.500	0.000 0.500
A2 Conc. (ng/ul)	0.386 - 0.904	0.667 - 1.000**	- 0.500	0.667 1.000	0.667 - 1.000**	0.667 1.000**	0.667 - 1.000**	0.667 1.000**	0.667 0.500	0.667 - 1.000**
A3 Purity	0.281 0.904	0.000 1.000**	0.667 0.500	- 1.000**	0.000 1.000	0.000 - 1.000**	0.000 1.000**	0.000 - 1.000**	0.667 -0.500	0.000 1.000**
A3 Conc. (ng/ul)	0.281 - 0.904	0.000 - 1.000**	0.667 - 0.500	0.000 1.000**	- 1.000**	0.000 1.000	0.000 - 1.000**	0.000 1.000**	0.667 0.5000	0.000 - 1.000**
A Ctrl Purity	0.281 0.904	0.000 1.000**	0.667 0.500	0.000 - 1.000**	0.000 1.000**	- 1.000**	0.000 1.000	0.000 - 1.000**	0.667 - 0.5000	0.000 1.000**
A Ctrl Conc. (ng/ul)	0.281 - 0.904	0.000 - 1.000**	0.667 - 0.500	0.000 1.000**	0.000 - 1.000**	0.000 1.000**	- 1.000**	0.000 1.000	0.667 0.5000	0.000 - 1.000**
Blank Ctrl Purity	0.281 - 0.082	0.000 -0.500	0.667 0.500	0.000 0.500	0.000 -0.500	0.000 0.500	0.000 -0.500	0.500	0.667 1.000	0.000 -0.500
Blank Conc. (ng/ul)	0.948 0.904	0.667 1.000**	0.667 0.500	0.667 - 1.000**	0.667 1.000**	0.667 - 1.000**	0.667 1.000**	0.667 - 1.000**	-0.500	0.667 1.000
	0.281	0.000	0.667	0.000	0.000	0.000	0.000	0.000	0.667	

**.correlation is significant at 0.01 level

Legend

A1= Clay soil/formaldehyde treatment group
A2= Clay soil/methanol treatment group
A3= Clay soil/pine oil treatment group
A Ctrl = A control
A Blk = A Blank Control group

Table 4 presents the Pearson correlation analysis within group B. The results showed that B1 purity was positively correlated with purity levels in B3, B positive control, B blank control, and B3 concentration level. Additionally, B2 concentration levels were positively correlated with concentration levels in the blank control, while B3 purity was positively correlated with concentration values in B3 and positive control, as well as purity values in B1 and blank control ($p \leq 0.01$).

Table 4: Pearson Correlation Analysis Output of Spectrophotometry Values-GROUP B

	B1 Purity	B1 Conc. (ng/ul)	B2 Purity	B2 Conc. (ng/ul)	B3 Purity	B3 Conc. (ng/ul)	B Ctrl Purity	B Ctrl Conc. (ng/ul)	B Blank Ctrl Purity	B Blank Ctrl Conc. (ng/ul)
B1 Purity	1.000	0.982	-0.5000	0.500	-1.000**	1.000**	1.000**	-1.000**	-1.000**	0.5000
B1 Conc. (ng/ul)		0.121	0.667	0.667	0.000	0.000	0.000	0.000	0.000	0.667
	0.982	1.000	-0.327	0.655	-0.982	0.982	0.982	-0.982	-0.982	0.655
B2 Purity	0.121		0.788	0.546	0.121	0.121	0.121	0.121	0.121	0.546
	-0.500	-0.327	1.000	0.500	0.500	-0.500	-0.500	0.500	0.500	0.500
B2 Conc. (ng/ul)	0.667	0.788		0.667	0.667	0.667	0.667	0.667	0.667	0.667
	0.500	0.655	0.500	1.000	-0.500	0.500	0.500	-0.500	-0.500	1.000**
B3 Purity	0.667	0.546	0.667		0.667	0.667	0.667	0.667	0.667	0.667
	-1.000**	-0.982	0.500	-0.500	1.000	-1.000**	-1.000**	1.000**	1.000**	-0.500
B3 Conc. (ng/ul)	0.000	0.121	0.667	0.667		0.000	0.000	0.000	0.000	0.667
	1.000**	0.982	-0.500	0.500	-1.000**	1.000	1.000**	-1.000**	-1.000**	0.500
B Ctrl Purity	0.000	0.121	0.667	0.667	0.000		0.000	0.000	0.000	0.667
	1.000**	0.982	-0.500	0.500	-1.000**	1.000**	1.000	-1.000**	-1.000**	0.500
B Ctrl Conc. (ng/ul)	0.000	0.121	0.667	0.667	0.000	0.000		0.000	0.000	0.667
	-1.000**	-0.982	0.500	-0.500	1.000**	-1.000**	-1.000**	1.000	1.000**	-0.500
B Blank Ctrl Purity	0.000	0.121	0.667	0.667	0.000	0.000	0.000		0.000	0.667
	-1.000**	-0.982	0.500	-0.500	1.000**	-1.000**	-1.000**	1.000**	1.000	-0.500
B Blank Conc. (ng/ul)	0.000	0.121	0.667	0.667	0.000	0.000	0.000	0.000		0.667
	0.500	0.655	0.500	1.000**	-0.500	0.500	0.500	-0.500	-0.500	1.000
		0.667	0.546	0.667	0.667	0.667	0.667	0.667	0.667	

**.correlation is significant at 0.01 level

Legend

B1= Clay soil/formaldehyde treatment group
B2= Clay soil/methanol treatment group
B3= Clay soil/pine oil treatment group
B Ctrl = B control
B Blk = B Blank Control group

Table 5 displays the correlation analysis between DNA concentration and purity within the C group. The purity level in C1 was positively correlated with concentration levels in C1, C2, positive control, and blank control, as well as purity levels in C2, C3, and positive control ($p \leq 0.01$). Furthermore, the concentration level in C1 was positively correlated with purity levels in C1, C2, C3, positive control, and concentration levels in positive control and blank control ($p \leq 0.01$). Additionally, the purity level in C2 was positively correlated with purity levels in C3, positive control, and concentration levels in C2 and blank control ($p \leq 0.01$). Finally, the concentration level in C2 was positively correlated with purity levels in C3, positive control, and concentration levels in positive control and blank control ($p \leq 0.01$).

Table 5: Pearson Correlation Analysis Output of Spectrophotometry Values-GROUP C

	C1 Purity	C1 Conc. (ng/ul)	C2 Purity	C2 Conc. (ng/ul)	C3 Purity	C3 Conc. (ng/ul)	C Ctrl Purity	C Ctrl Conc. (ng/ul)	C Blank Ctrl Purity	C Blank Ctrl Conc. (ng/ul)
C1 Purity	1.000	-1.000**	-1.000**	-1.000**	-1.000**	0.982	-1.000**	-1.000**	-0.866	1.000**
C1 C onc. (ng/ul)		0.000	0.000	0.000	0.000	0.121	0.000	0.000	0.333	0.000
	-1.000**	1.000	1.000**	1.000**	1.000**	-0.982	1.000**	1.000**	0.866	-1.000**
C2 Purity	0.000		0.000	0.000	0.000	0.121	0.000	0.000	0.333	0.000
	-1.000**	1.000**	1.000	1.000**	1.000**	-0.982	1.000**	1.000**	0.866	-1.000**
C2 Conc. (ng/ul)	0.000	0.000		0.000	0.000	0.121	0.000	0.000	0.333	0.000
	-1.000**	1.000**	1.000**	1.000	1.000**	-0.982	1.000**	1.0000**	0.866	-1.000**
C3 Purity	0.000	0.000	0.000		0.000	0.121	0.000	0.000	0.333	0.000
	-1.000**	1.000**	1.000**	1.000**	1.000	0.982	1.000**	1.0 00**	0.866	-1.000**
C3 Conc. (ng/ul)	0.000	0.000	0.000	0.000		0.121	0.000	0.000	0.333	0.000
	0.982	-0.982	-0.982	-0.982	-0.982	1.000	-0.982	-0.982	-0.756	0.982
C Ctrl Purity	0.121	0.121	0.121	0.121	0.121		0.121	0.121	0.454	0.121
	-1.000**	1.000**	1.000 **	1.000**	1.000**	-0.982	1.000	1.000**	0.866	-1.000**
C Ctrl Conc. (ng/ul)	0.000	0.000	0.000	0.000	0.000	0.121		0.000	0.333	0.000
	-1.000**	1.000**	1.000**	1.000**	1.000**	-0.982	1.000**	1.000	0.866	-1.000**
C Blank Ctrl Purity	0.000	0.000	0.000	0.000	0.000	0.121	0.000		0.333	0.000
	-0.866	0.866	0.866	0.866	0.866	-0.756	0.866	0.866	1.000	-0.866
C Blank Conc. (ng/ul)	0.333	0.333	0.333	0.333	0.333	0.454	0.333	0.333		0.333
	1.000**	-1.000**	-1.000**	-1.000**	-1.000**	0.982	-1.000**	-1.000**	-0.866	1.000
	0.000	0.000	0.000	0.000	0.000	0.121	0.000	0.000	0.333	

**.correlation is significant at 0.01 level

Legend

C1= Clay soil/formaldehyde treatment group
C2= Clay soil/methanol treatment group
C3= Clay soil/pine oil treatment group
C Ctrl = C control
C Blk = C Blank Control group

QUALITATIVE DECOMPOSITION ANALYSIS

After the exhumation of the corpses, a qualitative examination was conducted on the samples to determine the level of decay. The observed characteristics included the presence of skin corruption, loss of tissue, hooves disarticulation, adipocere formation, and decomposing hair. These features were recorded in a tabular format, with a positive sign (+) indicating the presence of the trait and a negative sign (-) denoting its absence (Table 6). The members of Group A, which consisted of clay soil, displayed a notable amount of skin corruption, complete loss of muscular tissue, hooves disarticulation, and the presence of decomposing hair. Among the subgroups in Group A, A2, which was subjected to methanol treatment, exhibited patches of adipocere formation. The B group, which comprised sandy soil, showed significant skin corruption, loss of tissues, and hooves disarticulation. Adipocere formation was not observed in any of the samples from this group. Despite being subjected to formaldehyde and methanol treatments, patches of decomposing hair were still present in some of the samples from this group.

The C group, consisting of loamy soil, exhibited complete decomposition of skin and muscle tissues, resulting in the disarticulation of bones and hooves. In all the treatment groups, except for the control group, patches of decomposing hair were observed. Furthermore, the methanol-treated samples displayed adipocere formation on the remnants of decomposing bones. A cross-sectional view of putrefied bones from the buried pig limbs after 12 months is illustrated in Figure 2.

Table 6: Qualitative decomposition analysis of exhumed pig limb carcasses

Soil type	Skin Corruption	Loss of tissue	Hooves disarticulation	Bone disarticulation	Adipocere Formation	Presence of Decomposing Hair
			A Group (Clay)			
A1	+	+	+	+	-	+
A2	+	+	+	+	-	+
A3	+	+	+	+	+	+
A CONTROL	+	+	+	+	-	+
			B Group (Sandy)			
B1	+	+	+	+	-	+
B2	+	+	+	+	-	+
B3	+	+	+	+	-	-
B CONTROL	+	+	+	+	-	-
			C Group (loamy)			
C1	+	+	+	+	-	+
C2	+	+	+	+	+	+
C3	+	+	+	+	-	+
C CONTROL	+	+	+	+	-	-

LEGEND: + = Present - = Absent

Weight Changes

To assess the degree of tissue loss and bone decomposition, the pre-burial and post-burial weights of the pig limbs used in the study were measured (Figure 3, 4, and 5). In all the groups, there was a noticeable reduction in post-burial weight compared to pre-burial weight. Upon exhumation, there was no preservation of skin or soft tissues, although the hard tissue (bones) was well-preserved.

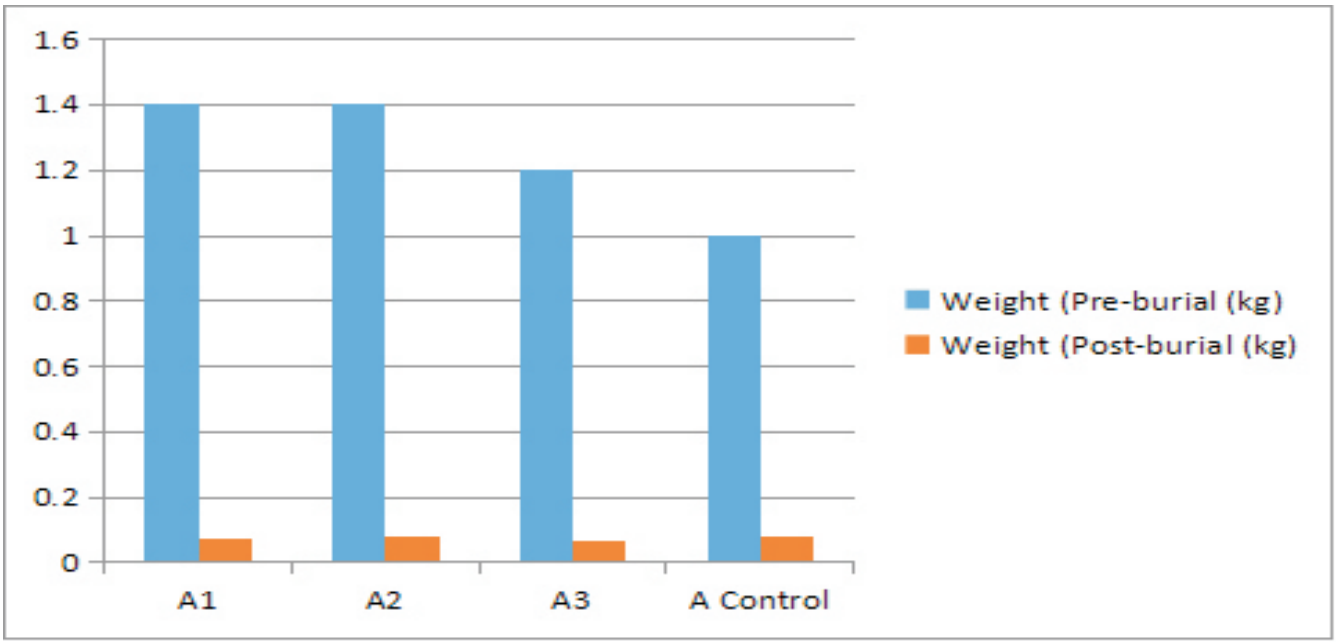

Figure 3: Change in the pre and post-burial weight of buried limbs in clay soil

LEGEND
A1= Clay Soil/ Formaldehyde Treatment Group
A3= ClaySoil/Pine Oil Treatment Group A2=ClaySoil/ Methanol Treatment Group

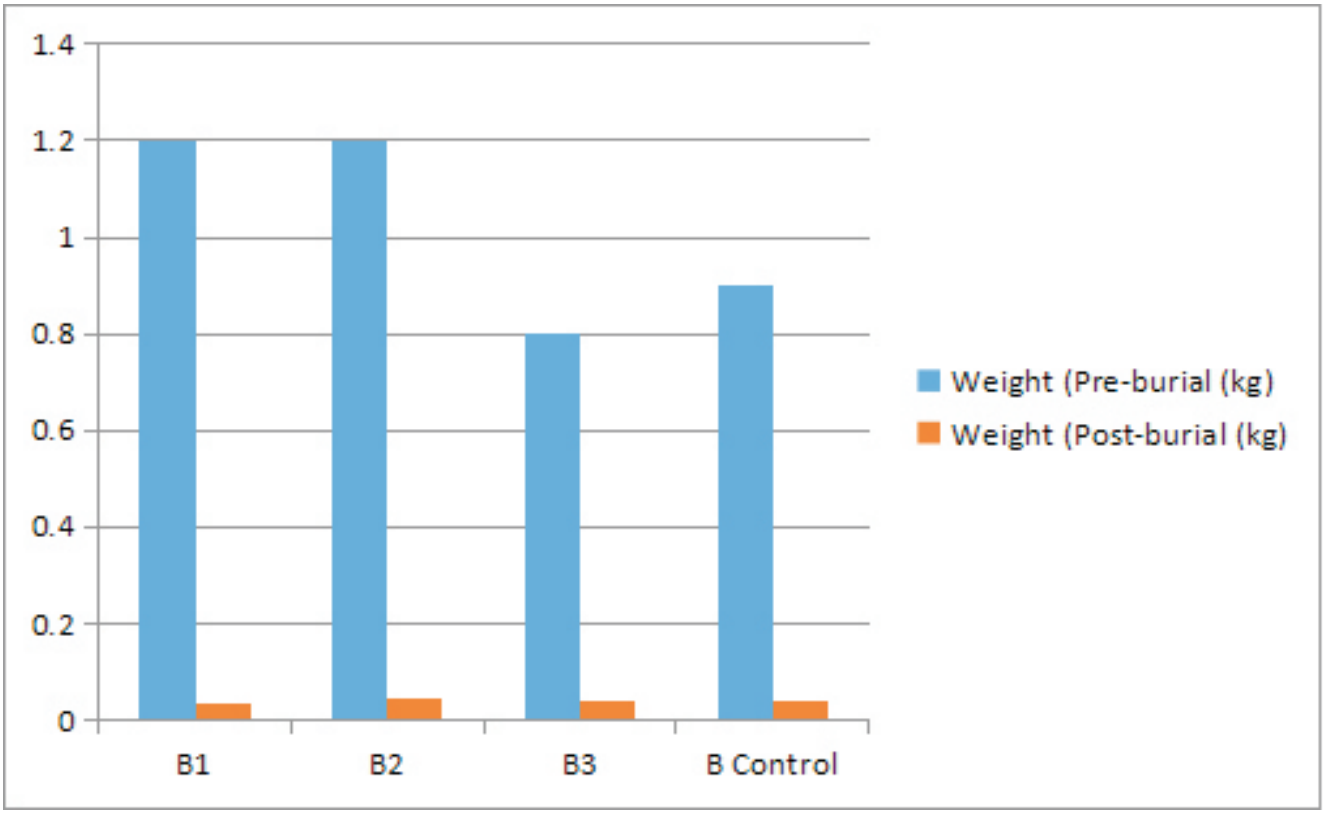

Figure 4: Change in the pre- and post-burial weight of buried limbs in sandy soil

LEGEND: B1= SandySoil/Formaldehyde Treatment Group

B2= SandySoil/Methanol Treatment Group B3= SandySoil/Pine Oil Treatment Group

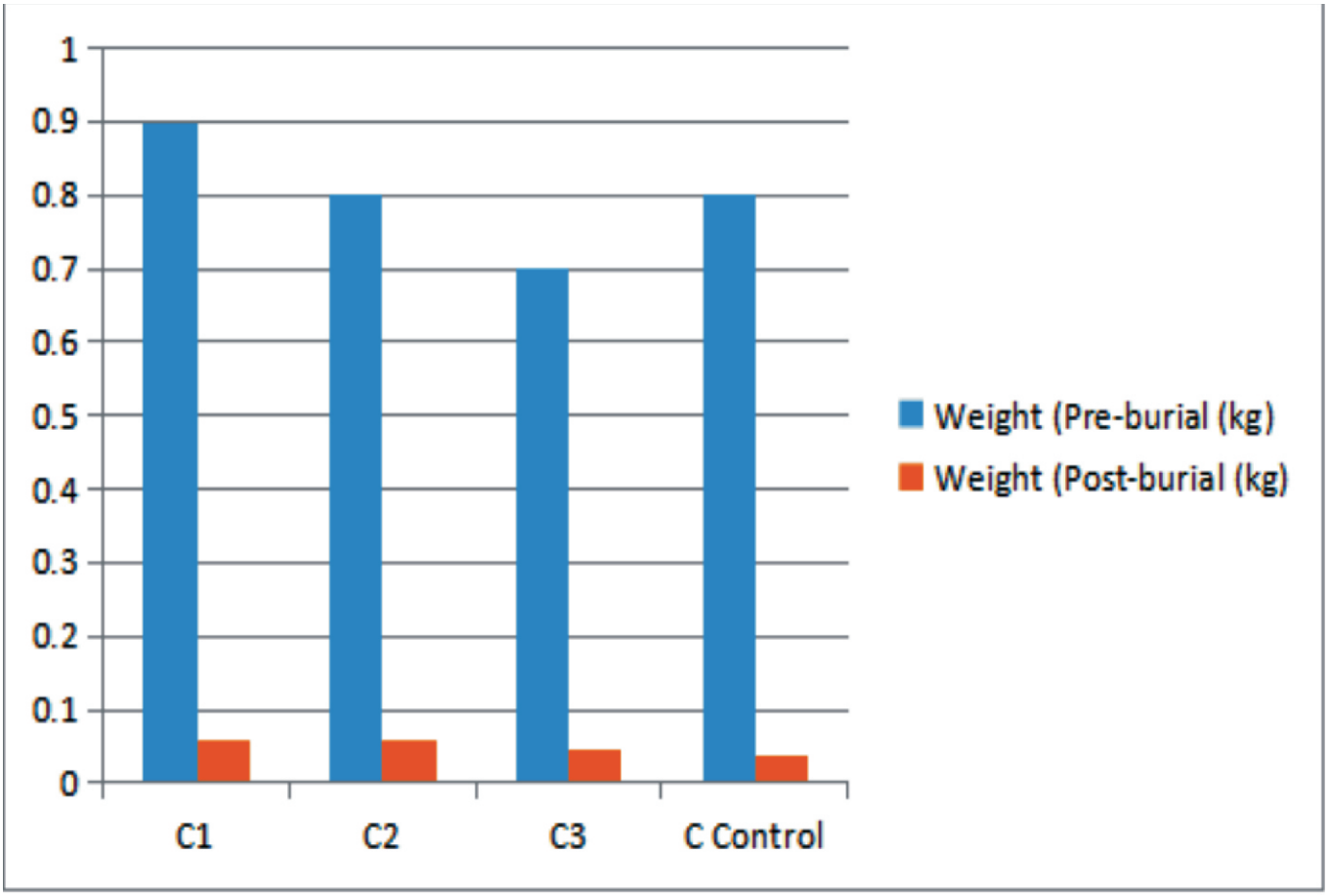

Figure 5: Change in the pre and post-burial weight of buried limbs in loam soil

LEGEND:
C1= LoamySoil/Formaldehyde Treatment Group
C2= LoamySoil/Methanol Treatment Group
C3= LoamySoil/Pine Oil Treatment Group

SOIL ENZYME ANALYSIS

The levels of Urease, Alkaline Phosphatase, Dehydrogenase, and $CaCO_3$ in the soil were analyzed descriptively, and the results are presented in Table 7. As per the table, the mean value ± SEM of Urease was highest in the grave soil A1 (240.67 ± 1.15 µmol NH4 h^{-1} g^{-1}), whereas the lowest level (148.75 ± 1.15 µmol NH4 h-1 g-1) was found in the blank control sample. The A positive control sample exhibited the highest Alkaline Phosphatase level (2278.27 ± 1.15 µmol/min/g) mean ± SEM, whereas the A3 sample (516.37 ± 1.15 µmol/min/g) showed the lowest value. Regarding Dehydrogenase enzyme activity, the A1 grave soil sample had the highest level (47.37 ± 1.15 µmol TFF kg^{-1}) mean ± SEM, whereas the A positive control showed the lowest value (21.74 ± 1.15 µmol TFFkg^{-1}). The highest $CaCO_3$ level (31.69 ± 1.15 %) mean ± SEM was observed in the A2 sample soil, while the lowest value (19.20 ± 1.15 %) was found in the A blank control.

Table 7: Descriptive Statistics of Levels of Soil Enzyme Assay in Group A

Groups	Parameters	Minimum	Maximum	Mean ± SE
	Urease (µmol NH4 h^{-1} g^{-1})	238.67	242.67	240.67±1.15
	Alkaline Phosphatase (µmol/min/g)	1606.63	1610.63	1608.63±1.15
A1	Dehydrogenase (µmol TFF kg- 1)	45.37	49.37	47.37±1.15
	$CaCO_3$ (%)	16.40	20.40	18.28±1.16
	Urease (µmol NH4 h-1 g-1)	156.77	160.77	158.77±1.154
	Alkaline Phosphatase (µmol/min/g)	1161.69	1165.69	1163.69±1.15
A2	Dehydrogenase (µmol TFF kg- 1)	45.05	49.05	47.05±1.15
	$CaCO_3$ (%)	29.69	33.69	31.69±1.15
	Urease (µmol NH4 h-1 g-1)	216.94	220.94	218.94±1.15
	Alkaline Phosphatase (µmol/min/g)	514.37	518.37	516.37±1.15
A3	Dehydrogenase (µmol TFF kg- 1)	41.41	45.41	43.41±1.15
	$CaCO_3$ (%)	28.19	32.19	30.19±1.15
	Urease (µmol NH4 h-1 g-1)	146.75	150.75	148.75±1.15
Blank	Alkaline Phosphatase (µmol/min/g)	1544.13	1548.13	1546.13±1.15
Control	Dehydrogenase (µmol TFF kg- 1)	44.70	48.70	46.70±1.15
	$CaCO_3$ (%)	17.20	21.20	19.20±1.15
	Urease (µmol NH4 h-1 g-1)	185.19	189.19	187.19±1.15
Positive	Alkaline Phosphatase (µmol/min/g)	2276.27	2280.27	2278.27±1.15
Control	Dehydrogenase (µmol TFF kg- 1)	19.74	23.74	21.74±1.15
	$CaCO_3$ (%)	17.50	21.50	19.50±1.15

Table 8 displays the descriptive statistics of the levels of the determined soil enzymes in the B group. Within the group, the highest soil level urease (310.86 ± 1.15 µmol NH4 h-1 g-1) mean ± SEM was found in the B1 sample soil, while the lowest value (53.48 ± 1.15 µmol NH4 h-1 g-1) was observed in the blank control sample. In contrast to the other enzymes, the highest soil level of Alkaline Phosphatase (2138.39 ± 1.15 µmol/min/g) mean ± SEM was found in the blank control sample within the B group, whereas the lowest level (184.52 ± 1.15 µmol/min/g) was recorded in the B3 sample grave soil. The B group showed that the soil level dehydrogenase was highest in the blank control group (45.55 ± 1.15 µmol TFF kg−1) mean ± SEM, while it was lowest in the B1 soil sample (21.65 ± 1.15 µmol TFF kg−1). Within the B group, the soil level $CaCO_3$ was highest in the B1 sample (30.75 ± 1.15 %) mean ± SEM and lowest in the B blank control sample (19.20 ± 1.15 %).

Table 8: Descriptive Statistics of Levels of Soil Enzyme in Group B

Groups	Parameters	Minimum	Maximum	Mean ± SEM
	Urease (µmol NH4 h-1 g-1)	308.86	312.86	310.86 ± 1.15
B1	Alkaline Phosphatase (µmol/min/g)	197.40	201.40	199.40 ±1.15
	Dehydrogenase (µmol TFF kg- 1)	19.65	23.65	21.65 ±1.15
	$CaCO_3$ (%)	28.75	32.75	30.75 ±1.15
	Urease (µmol NH4 h-1 g-1)	220.28	224.28	222.28 ±1.15
B2	Alkaline Phosphatase (µmol/min/g)	185.50	189.50	187.50 ±1.15
	Dehydrogenase (µmol TFF kg- 1)	21.20	25.20	23.20 ±1.15
	$CaCO_3$ (%)	26.43	30.43	28.43 ±1.15
	Urease (µmol NH4 h-1 g-1)	186.86	200.86	192.19± 4.37
B3	Alkaline Phosphatase (µmol/min/g)	182.52	186.52	184.52 ±1.15
	Dehydrogenase (µmol TFF kg- 1)	20.18	24.18	22.18 ±1.15
	$CaCO_3$ (%)	14.59	18.59	16.59 ±1.15
Positive	Urease (µmol NH4 h-1 g-1)	130.03	134.03	132.03 ±1.15
Control	Alkaline Phosphatase (µmol/min/g)	1069.43	1073.43	1071.43±1.15
	Dehydrogenase (µmol TFF kg- 1)	34.16	38.16	36.16 ±1.15
	$CaCO_3$ (%)	30.38	34.38	32.38 ±1.15
Blank	Urease (µmol NH4 h-1 g-1)	51.48	55.48	53.48 ±1.15
control	Alkaline Phosphatase (µmol/min/g	2136.39	2140.39	2138.39±1.15
	Dehydrogenase (µmol TFF kg- 1)	43.55	47.55	45.55 ±1.15
	$CaCO_3$ (%)	17.20	21.20	19.20 ±1.15

Table 9 presents the descriptive statistical analysis of the levels of soil enzymes in study Group C. The highest soil urease level was recorded in the C1 sample, with a Mean ± SEM value of 329.25 ± 1.15 µmol NH_4 h^{-1} g^{-1}, while the lowest level was observed in the blank control soil sample, with a Mean ± SEM value of 86.91 ± 1.15 µmol NH4 h^{-1} g^{-1}. The C blank sample had the highest Alkaline phosphatase level (2226.19 ± 1.15 µmol/min/g) Mean ± SEM within the group, whereas the lowest level was observed in the C1 grave soil sample (139.88 ± 1.15 µmol/min/g). The positive control group had the highest soil level dehydrogenase (49.34 ± 1.15 µmol TFF kg^{-1}) Mean ± SEM, whereas the blank control group had the least (33.64 ± 1.15 µmol TFF kg^{-1}). Regarding the $CaCO_3$ level, the highest was observed in the positive control group (32.42 ± 1.15 %) Mean ± SEM while the lowest was recorded in the C1 sample (17.86 ± 1.15 %).

Table 9: Descriptive Statistics of Levels of Soil Enzyme in Group C

Groups	Parameters	Minimum	Maximum	Mean ± SEM
	Urease (µmol NH4 h-1 g-1)	327.25	331.25	329.25±1.15
C1	Alkaline phosphatase (µmol/min/g)	137.88	141.88	139.88±1.15
	Dehydrogenase (µmol TFF kg- 1)	33.27	37.27	35.27±1.15
	$CaCO_3$ (%)	15.86	19.86	17.86±1.15
	Urease (µmol NH4 h-1 g-1)	235.33	239.33	237.33±1.15
C2	Alkaline phosphatase (µmol/min/g)	130.44	134.44	132.44±1.15
	Dehydrogenase (µmol TFF kg- 1)	33.05	37.05	35.05±1.15
	$CaCO_3$ (%)	17.01	21.01	19.01±1.15
	Urease (µmol NH4 h-1 g-1)	73.21	77.21	75.21±1.15
C3	Alkaline phosphatase (µmol/min/g)	137.88	141.88	139.88±1.15
	Dehydrogenase (µmol TFF kg- 1)	29.57	33.57	31.57±1.15
	$CaCO_3$ (%)	17.52	21.52	19.52±1.15
	Urease (µmol NH4 h-1 g-1)	145.08	149.08	147.08±1.15
Positive	Alkaline Phosphatase (µmol/min/g)	1375.98	1379.98	1377.98±1.15
Control	Dehydrogenase (µmol TFF kg- 1)	47.34	51.34	49.34±1.15
	$CaCO_3$ (%)	30.42	34.42	32.42±1.15
	Urease (µmol NH4 h-1 g-1)	84.91	88.91	86.91±1.15
Blank	Alkaline Phosphatase (µmol/min/g)	2224.19	2228.19	2226.19±1.15
Control	Dehydrogenase (µmol TFF kg- 1)	31.64	35.64	33.64±1.15
	$CaCO^3$ (%)	29.29	33.29	31.29±1.15

STATISTICAL CORRELATION ANALYSIS OF SOIL ENZYME ASSAY

All experimental grave soil correlated with the blank and positive control grave soil samples**.**

MONTHLY AVERAGE TEMPERATURE, HUMIDITY AND RAINFALL FOR THE EXPOSURE PERIOD

Table 10 shows the monthly average temperature, humidity and rainfall for the period of study.

Table 10: Monthly average temperature, humidity and rainfall for the burial period

Month	Average Temperature (^oC)	Average Humidity (%)	Average Rainfall (mm)
June (2020)	28.1	7.79	33.1
July	28.6	7.37	0
August	30.1	7.43	0
September	28.8	7.25	11.6
October	30.4	8.47	120.5
November	31.2	7.24	0
December	31.7	7.29	0
January (2021)	30.7	7.20	0
February	31.0	7.50	0
March	28.5	8.0	57.5
April	31.1	7.92	17.19
May	30.6	7.57	18.8
June (2021)	29.5	8.18	33.1

DISCUSSION

Forensic taphonomy is a field of study that examines the effects of post-mortem processes on the preservation, detection, and recovery of human remains (Martin et al. 2021). The process of decomposition gradually breaks down a corpse into smaller components that are eventually recycled back into the environment. The rate and extent of decomposition are influenced by various factors such as soil properties, pH, accessibility to insects, microbial activity, as well as pre-existing factors such as age, weight, and any chemical treatments the body may have undergone before death. Forensic taphonomy studies these changes to help with investigations, estimating the time since death, and locating hidden graves (Hau et al. 2014).

In recent years, various scientific methods have been published in the literature for using DNA to detect the post-mortem interval (PMI) (Williams et al. 2015). Various methods for assessing nuclear DNA fragmentation for postmortem interval (PMI) detection have been explored, including both qualitative and quantitative techniques. In recent decades, DNA amplification methods like Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) have also been used (Shukla, 2017). In this study, the modified DNA salting-out protocol by (Medrano et al. 1990) was adopted and the amount of DNA yield was measured using the spectrophotometer. Significant decrease in the concentration and purity was observed in Clay soil and Loamy soil groups when compared to the control samples. Also, decrease in concentration and purity was recorded in Sandy soil group but was not statistically significant when compared to the control samples except the Sandy/Methanol treatment sample that recorded a significantly reduced DNA concentration. Overall, the yield of the isolated DNA was very low most likely due to the 365 days PMI. This finding is consistent with the finding of Mansour et al. 2019 which showed that the shortest postmortem interval (PMI) showed the highest amount of DNA, and the concentration of DNA decreased significantly after 10 days following death. Their study demonstrated that high DNA concentrations were obtained from burnt and fresh corpses with PMI not exceeding 1-2 days, even though PMI was confounded with conditions such as post-mortem condition and surrounding environment. Furthermore, gel electrophoresis of the PCR amplified DNA showed no bands. This finding maybe due to the very low DNA yield from our samples and our chosen DNA extraction protocol as a modified FFPE tissue extraction protocol used by (Gielda et al. 2017) yielded high output DNA readily amplified on gel electrophoresis.

Decomposition rates and net mineralization rely on soil organisms' access to organic substrates (Mouhamad et al. 2015). Soil enzymes play a vital role in catalyzing various biochemical processes that lead to the transformation of organic materials in the soil. These processes ultimately result in the release of inorganic nutrients, which are essential for plant development and nutrient cycling in the ecosystem (Baležentienė, 2012). These soil enzymes are closely related to enzymes that directly interact with dead bodies and plant materials. They can provide valuable information about specific characteristics of the microbiome present in the soil. By studying these enzymes, researchers can gain insights into the decomposition processes, nutrient cycling, and microbial activity associated with dead bodies and plant materials in the soil environment (Fioretto et al. 2000).

In a study conducted by (Tumer et al. 2013), it was observed that the extensive tissue decomposition of pig extremities buried in various soil particles resulted in the production of ammonia (NH_3) from the decomposing tissues. This release of ammonia is a significant component of nitrogen cycling in the soil. Additionally, the study found a correlation between the degree of tissue decomposition and the soil urease level, suggesting a relationship between the decomposition process and the activity of urease enzyme in the soil. Urease activity has been found to increase under organic fertilization, demonstrating the tight connection between soil organic matter and nitrogen cycling (Mohammadi, 2011). In our study, it was observed that increase in soil urease was recorded in sandy and loamy grave soils while a decrease was observed in the clay grave soils. This finding contradicts the results reported by Tumer et al. (2013), where the lowest urease activity was observed in sandy grave soil during their decomposition study.

Also, phosphatases are a crucial group of soil enzymes (Telesiński et al. 2018). They have a significant role in the biochemical mineralization of organic phosphorus, making them valuable

indicators of the potential for organic phosphorus mineralization and the biological activity occurring in the soil (Lemanowicz et al. 2016). In the present study, our hypothesis was that there would be a distinct difference in soil properties between the grave soils and control soils due to the extensive decomposition that had occurred. However, contrary to our expectations, we found that the experimental grave soils exhibited a reduced level of alkaline phosphatase compared to the control soils. This finding aligns with the results reported by Tumer et al. (2013), which also observed no significant difference in soil level alkaline phosphatase between the control and grave soils.

Furthermore, dehydrogenases are one of the most important groups of soil enzymes. They play a crucial role in the biological oxidation of organic matter in soil. It facilitates the transfer of hydrogen from an organic substrate to inorganic acceptors as part of the process of organic matter degradation (Strek and Telesinski, 2015). The activity of dehydrogenase is considered an indicator of the oxidative metabolism in soils and thus of the microbial activity (Kaczyńska et al. 2015). Dehydrogenase is an intracellular enzyme that is primarily associated with viable cells. In the current study, we observed a significant decrease in soil dehydrogenase levels in all grave soils compared to the control soils. This finding contradicts our initial hypothesis, particularly considering previous research by (Quilchano and Marañón, 2002), which reported that soil clay content tends to increase enzyme activity. Our study's results suggest that the burial process and associated decomposition may have a negative impact on soil dehydrogenase activity, leading to reduced enzyme levels in the grave soils. According to a previous study by (Vass et al. 1992), during the process of decomposition, inorganic substances like calcium, magnesium, and others are released from the decaying organic material. Additionally, there is a positive correlation between dehydrogenase activity and $CaCO_3$ levels, as well as between organic matter content and dehydrogenase activity. The spatial distribution of dehydrogenase activity, organic matter, and $CaCO_3$ suggests that a higher concentration of organic matter promotes the formation of $CaCO_3$ (Zhang et al. 2010). These findings indicate the interplay between organic matter, dehydrogenase activity, and the formation of $CaCO_3$ during the decomposition process. Contrary to the previously mentioned research findings, the soil level of $CaCO_3$ showed a significant decrease in all experimental grave soils, except for the clay/formaldehyde grave soil, when compared to the control soils. This finding indicates that the process of decomposition in the experimental grave soils has led to a reduction in $CaCO_3$ levels, which is contrary to the expected correlation between organic matter content and $CaCO_3$ formation.

Overall, in this study, we examined various metrics for assessing decomposition, estimating PMI, and detecting post-mortem chemical exposure in criminal investigations. It found that qualitative measures of decomposition were correlated with pre- and post-burial weight changes, but there was no direct link between embalming chemicals, soil type, and decomposition rate. Our study also revealed some contrasting results with previous research on the relationship between organic matter decomposition and soil biochemical properties, which might be due to the use of embalming chemicals. Additionally, the absence of bands on gel electrophoresis could be due to very low DNA yield from the samples or the non-stringency of the used primers.

CONCLUSION

When conducting criminal investigations and verifying witness testimonies, it is important to take into account various factors that can affect the decomposition process and the determination of post-mortem interval (PMI). This includes analyzing the soil and biochemical properties of the burial grave, as well as considering any potential exposure to embalming chemicals before death. By taking these factors into consideration, investigators can better evaluate the decomposition process and accurately determine PMI, which can ultimately aid in the timely dispensation of justice.

REFERENCES

Baležentienė L. (2012). Hydrolases Related to C and N Cycles and Soil Fertility Amendment: Responses to Different Management Styles of Agro-Ecosystems. Polish Journal of Environmental Studies. Polish Journal of Environmental Studies. 21(5): 1153-1159.

Balta JY, Twomey M, Moloney F, Duggan O, Murphy KP, O'Connor OJ, Cronin M, Cryan JF, Maher MM,

O'Mahony SM. (2019). A comparison of embalming fluids on the structures and properties of tissue in human cadavers. Anatomia histologia embryologia. 48(1): 64–73. https://doi.org/10.1111/ahe.12412

Fioretto A, Papa S, Curcio E, Sorrentino G, Fuggi A. (2000). Enzyme dynamics on decomposing leaf litter of Cistus incanus and Myrtus communis in a Mediterranean ecosystem. Soil Biology and Biochemistry. 32(13): 1847-1855. https://doi.org/10.1016/S0038-0717(00)00158-9

Gielda L, Rigg S. (2017). Extraction of amplifiable DNA from embalmed human cadaver tissue. BMC research notes. 10(1): 1-5. https://doi.org/10.1186/s13104-017-3066-y

Hau TC, Hamzah NH, Lian HH, Hamzah S. (2014). Decomposition process and post mortem changes. Sains Malaysiana. 43(12): 1873-1882. https://doi.org/10.17576/jsm-2014-4312-08

Hebert PD, Cywinska A, Ball SL, deWaard JR. (2003). Biological identifications through DNA barcodes. Proceedings. Biological Sciences. 270(1512): 313-321. https://doi.org/10.1098/rspb.2002.2218

Kaczyńska G, Borowik A, Wyszkowska J. (2015). Soil dehydrogenases as an indicator of contamination of the environment with petroleum products. Water, Air, and Soil Pollution. 226: 1-11. https://doi.org/10.1007/s11270-015-2642-9

Kandeler E, Kampichler C, Horak O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. Biol Fertil Soils. 23: 299–306. https://doi.org/10.1007/BF00335958

Lee PY, Costumbrado J, Hsu CY, Kim YH. (2012). Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments, JoVE. (62): 3923. https://doi.org/10.3791/3923

Lemanowicz J, Bartkowiak A, Breza-Boruta B. (2016). Changes in phosphorus content, phosphatase activity and some physicochemical and microbiological parameters of soil within the range of impact of illegal dumping sites in Bydgoszcz (Poland). Environmental Earth Sciences. 75: 1-14. https://doi.org/10.1007/s12665-015-5162-4

Martin C, Maesen P, Minchilli D, Francis F, Verheggen F. (2021). Forensic taphonomy: Characterization of the gravesoil chemistry using a multivariate approach combining chemical and volatile analyses. Forensic Science International. 318: 110569. https://doi.org/10.1016/j.forsciint.2020.110569

Matuszewski S, Hall MJ, Moreau G, Schoenly KG, Tarone AM, Villet MH. (2020). Pigs vs people: the use of pigs as analogues for humans in forensic entomology and taphonomy research. International Journal of Legal Medicine. 134: 793-810. https://doi.org/10.1007/s00414-019-02074-5

Medrano JF, Aasen E, Sharrow L. (1990). DNA extraction from nucleated red blood cells. Biotechniques. 8(1): 43-43. http://www.ncbi.nlm.nih.gov/pubmed/2182076.

Mohammadi K. (2011). Soil microbial activity and biomass as influenced by tillage and fertilization in wheat production. American-Eurasian Journal of Agricultural and Environmental Science. 10(3): 330-337.

Mouhamad R, Atiyah A, Mohammad R, Iqbal, M. (2015). Decomposition of organic matter under different soil textures. Current Science Perspectives. 1(1): 22-25.

Naseby D, Lynch, J. (1997). Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of Pseudomonas fluorescens on wheat seed. Soil Biology and Biochemistry. 29(9-10): 1353-1362. https://doi.org/10.1016/S0038-0717(97)00061-8

Prangnell J, McGowan G. (2009). Soil temperature calculation for burial site analysis. Forensic Science International. 191(1-3): 104-109. https://doi.org/10.1016/j.forsciint.2009.07.002

Quilchano C, Marañón T. (2002). Dehydrogenase activity in Mediterranean forest soils. Biology and Fertility of Soils. 35: 102-107. https://doi.org/10.1007/s00374-002-0446-8

Shukla RK. (2017). Forensic application of comet assay: an emerging technique. Forensic Sciences Research. 2(4): 180-184. https://doi.org/10.1080/20961790.2017.1379893

Singh S, Yong SK, Rahimi R, Singh MKC, Heo CC. (2023). A review of forensic applications of physicochemical parameters of soil beneath decomposed cadavers. Canadian Society of Forensic Science Journal. 1-19. https://doi.org/10.1080/00085030.2023.2172129

Sinsabaugh R, Linkins A. (1988). Exoenzyme activity associated with lotic epilithon. Freshwater Biology. 20(2): 249-261.

Strek M, Telesinski A. (2015). Change in oxidoreductase activity of selected microbial enzymes in gasoline-contaminated light soil in presence of selenium. OCHRONA SRODOWISKA. 37(1): 43-47.

Tabatabai MA, Bremner JM. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry.* **1(4):** 301-307. https://doi.org/10.1016/0038-0717(69)90012-1

Telesiński A, Krzyśko-Łupicka T, Cybulska K., Wróbel J. (2018). Response of soil phosphatase activities to contamination with two types of tar oil. *Environmental Science and Pollution Research*. **25:** 28642-28653. https://doi.org/10.1007/s11356-018-2912-3

Thalmann A. (1968). Zur Methodik der Bestimmung der DehydrogenaseaktivitAt im Boden mittels triphenytetrazoliumchlorid (TTC). *Landwirtsch. Forsch.* ***21:*** 249-258.

Tibbett M, Carter DO, Haslam T, Major R, Haslam R. (2004). A laboratory incubation method for determining the rate of microbiological degradation of skeletal muscle tissue in soil. *Journal of Forensic Sciences.* ***49*****(3):** 560-565. https://doi.org/10.1520/JFS2003247

Tumer AR, Karacaoglu E, Namli A, Keten A, Farasat S, Akcan R, Sert O, Odabaşi, A. B. (2013). Effects of different types of soil on decomposition: An experimental study. *Legal medicine*, *15*(3), 149-156. https://doi.org/10.1016/j.legalmed.2012.11.003

Ubelaker DH. (2018). Recent advances in forensic anthropology. *Forensic Sciences Research.* ***3*****(4):** 275-277. https://doi.org/10.1080/20961790.2018.1466384

Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT. (1992). Time since death determinations of human cadavers using soil solution. *Journal of Forensic Science.* **37(5):** 1236-1253.

Williams T, Soni S, White J, Can G, Javan GT. (2015). Evaluation of DNA degradation using flow cytometry: promising tool for postmortem interval determination. *The American Journal of Forensic Medicine and Pathology.* ***36*****(2):** 104-110. https://doi.org/10.1097/PAF.0000000000000146

Zhang N, Xing-Dong H, Yu-Bao G, Yong-Hong L, Hai-Tao W, Di M, Zhang R, Yang S. (2010). Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in Artemisia ordosica community. *Pedosphere*. **20(2):** 229-235. https://doi.org/10.1016/S1002-0160(10)60010-0