



OPEN A comparative study of decomposition and associated insects on pig carcasses buried at 40 and 80 cm depths

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Forensic entomology is a branch of science that uses insects as evidence in medicolegal investigations as they could provide valuable information regarding the cause and time since death specially in the case of buried bodies. The present study was the first attempt in India to compare the decomposition and associated insects on pig carcasses above the ground and those buried at two different depths (40 and 80 cm) during spring season over two consecutive years. Two pigs were buried at each depth and one pig carcass was placed above the ground. One of the buried carcasses was exhumed frequently and other one was kept undisturbed to investigate the decomposition rate and insect access to the carcass. The rate of decomposition was found to be faster above the ground as compared to the buried environment. Also, the carcass buried at 80 cm depth exhibited slower decomposition rates and had limited insect activity as compared to the one buried at 40 cm. Species within the Calliphoridae and Sarcophagidae families were dominant on the surface-exposed carcass, whereas Phoridae were more prevalent on the buried carcasses even at a depth of 80 cm. The findings of the study highlights the significant influence of burial depth and exhumation on decomposition rate and insect arrival and colonization.

Keywords Entomofauna, Burial depth, Pig, Colonization, Spring, Decomposition, Frequent exhumation

The scientific study of human death and its effects on the environment is called forensic taphonomy which focuses on the decomposition process and the ecological influences that affect these changes¹. This field examines the decomposition of cadavers in various environments, including different burial conditions, and the role of external factors like temperature, soil composition, burial depth, and insect activity^{2,3}. In any homicide investigation, the precise estimation of postmortem interval (PMI) is very crucial. It refers to the period between death and subsequent retrieval of the dead body. There are numerous approaches for determining the postmortem interval that comprise early and late post-mortem changes. In cases where the bodies are retrieved in the advanced stages of decomposition wherein traditional methods fail in the assessment of PMI, forensic entomology can be very helpful in estimation of minimum postmortem interval (PMI_{min}) on and under the ground⁴. The PMI_{min} refers to the entomologically estimated time since death. It is based on the principle that insects typically associated with decomposing remains colonize a carcass just after death. Accordingly, assessing the age of the oldest immature insect specimen collected from the remains, in conjunction with the expected arrival time of adult females, provides an estimate of the minimum duration for which the remains have been available for insect colonization, thereby establishing the PMI_{min}⁵.

Forensic entomology is the scientific discipline that focuses on the use of insects in criminal investigations⁶. Flies are known to arrive and colonize the cadaver shortly after death⁷. In the course of their work, forensic entomologists encounter bodies in a variety of conditions. These include bodies that are found on the surface, buried, floating, burned, indoors or outdoors, wrapped, located in high-rise buildings, as well as in a variety of other situations⁸. In each situation, access of insects to the body may differ, so various influencing conditions are required to be taken into consideration when arriving at a PMI_{min}. Burial is one of the most used methods to conceal the body in homicidal cases. Perpetrators may choose burial as it can effectively hide evidence of the crime for an extended period, particularly if the grave is deep and well-hidden. Whereas shallow graves may be a practical choice in cases where time or resources are limited. Therefore, most clandestinely buried cadavers are found at various depths in a range of 30 to 90 cm⁹.

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The decomposition process, rate, and insect access in buried environments are influenced by various factors, including soil type, soil pH, temperature, humidity, and burial depth^{10,11}. Burial depth significantly impacts the accessibility of decomposing remains to insects; deeper burials may deter insect colonization, while shallow graves are more likely to be discovered by insects^{3,12}. The decomposition stages in the buried environment are similar to those on the surface, but the decomposition rate of cadavers in the graves is slower¹³. Soil covers may restrict insect accessibility in burial environments and, consequently, decrease the decomposition rate³. It should however be noted that graves will not be devoid of insects, so the study of succession patterns of associated insects may be used in the investigation of a crime¹⁴. Blowflies (Calliphoridae) are the early colonizers on the exposed cadavers¹⁵, but in the graves especially deep burials, these species may not have access to the carcass^{16–19}. In the buried environment the Phoridae, Sarcophagidae, and Muscidae are the most prominent ones followed by the coleopterans²⁰.

By understanding the unique conditions and challenges posed by burial, forensic scientists can develop better tools and techniques to aid in legal investigations and contribute to the broader understanding of decomposition processes²¹. Although the forensic entomology field has seen significant advancements on the terrestrial surface, research in the context of buried environments remained relatively underdeveloped. Therefore, the purpose of the present study was to examine the decomposition pattern, insect access, and colonization on the buried carcasses at two different depths as well as on the terrestrial surface in the spring season. The frequent exhumation of buried carcasses also has a considerable effect on the decomposition rate and insect diversity, so, another focus of the present study was to compare the decomposition rate and entomofauna associated with undisturbed and frequently exhumed buried carcasses.

Methods

Experimental site and experiment design

The present study was conducted at the Entomological facility at Maharshi Dayanand University, Rohtak during the spring season for two consecutive years (March to May 2022 and March to May 2023). Rohtak is a semi-arid region in Haryana with coordinates 28.8955° N, 76.6066° E. This site was chosen for the study because it is free from any human activity, and this is a bushy area with plenty of insect activity.

Slaughtered pigs (*Sus scrofa*) of similar weight with an average of 40 kg were purchased from the local slaughterhouse. Pig carcasses were covered with a tarpaulin sheet and immediately transported to the study site to avoid any invasion of the insects before the experiment started.

A total of ten pig carcasses were used for the present study. Two pairs of pigs were buried at 40 cm and 80 cm depth respectively and one was placed above the ground during the period of March to May 2022. The remaining five pigs were used in the replicated study (March to May 2023). An excavator (Manufacturer JCB excavator Ltd.) was used to dig burial pits at a depth of 40 and 80 cm each (with measurements of 1 × 0.76 m (length and breadth)) with a 10 m distance between them to avoid the cross interference of the insects. The pits were excavated 30 min before the burial of the carcasses to prevent early colonization of the graves by terrestrial insects. The pig carcasses were placed in the cages (dimensions 90 × 60 × 40 cm with mesh size 3 × 3 cm) and buried immediately after being placed in the burial pits. One pig carcass was exhumed frequently, whereas the other was left undisturbed, to compare their decomposition and the associated entomofauna at both the depths. The subaerially exposed carcasses were placed in cages with the same dimensions used for buried carcasses to avoid scavenger activity and allowed to decompose in the natural environment. To record the daily temperature and humidity data, a digital thermometer (Elitech-RC-4HC) was placed along with the pig carcasses on and under the ground. The experimental setup was the same for the replicated study. For understanding purposes, the pig carcasses have been coded as shown in Table 1.

The sampling of the buried carcass ($B_{40}EF_{2022}$) was conducted on days 8, 15, 22, 30, 45, 60, 70, and 90 at a depth of 40 cm. Similarly, at 80 cm depth ($B_{80}EF_{2022}$ carcass) the sampling was carried out on days 8, 15, 22, 29, 35, 50, 65, 80, 110, 120, 150, 180, 210, and 240, and the decomposition stages were observed accordingly. Throughout the exhumation process, the grave soil was continuously hand-sorted for the presence of entomofauna. The insect activity around the graves was also observed for the carcasses which were kept undisturbed. The carcass (T_{2022}) placed above the ground was monitored from morning to evening hours to observe the insect activity on

Sr. no.	Experimental model (pig) (codes)	Treatment with year
1.	$B_{40}EF_{2022}$	Buried at 40 cm and exhumed frequently (2022)
2.	$B_{40}EF_{2023}$	Buried at 40 cm and exhumed frequently (2023)
3.	$B_{40}EO_{2022}$	Buried at 40 cm and exhumed only once (2022)
4.	$B_{40}EO_{2023}$	Buried at 40 cm and exhumed only once (2023)
5.	$B_{80}EF_{2022}$	Buried at 80 cm and exhumed frequently (2022)
6.	$B_{80}EF_{2023}$	Buried at 80 cm and exhumed frequently (2023)
7.	$B_{80}EO_{2022}$	Buried at 80 cm and exhumed only once (2022)
8.	$B_{80}EO_{2023}$	Buried at 80 cm and exhumed only once (2023)
9.	T_{2022}	Subaerial exposure (2022)
10.	T_{2023}	Subaerial exposure (2023)

Table 1. Shows the codes given to the pig carcasses. B_{40} - Buried at 40 cm depth, B_{80} - buried at 80 cm depth, T- Terrestrial surface, EF- Exhumed frequently and EO- Exhumed once.

and around the carcass. The adult insects were collected with the help of nets and forceps and their immature stages were collected by using the brushes and forceps and preserved in 70% ethanol for further identification. Morphological keys were used to identify the insects^{22–25} and were also confirmed by the Zoological Survey of India (ZSI), Kolkata, India. The standard error for the average temperature and humidity above and below the ground was calculated using the IBM SPSS 23 statistical software.

Results

The average temperature and humidity (\pm S. E) recorded in the spring season was $31.8\text{ }^{\circ}\text{C} \pm 0.50$ and $40.6\% \pm 1.16$ respectively above the ground in both years. However, the average temperature in the graves was recorded as $22.8\text{ }^{\circ}\text{C} \pm 0.14$ and $20\text{ }^{\circ}\text{C} \pm 0.16$ respectively at 40 and 80 cm depth (Fig. 1). On the other hand, the average humidity in the buried environment was 40 ± 1.13 and $55\% \pm 0.64$ respectively at both depths (Fig. 2). The average rainfall recorded was 41.6 and 190.8 mm during 2022 and 2023 respectively.

Fresh, bloated, active decay, advanced decay and dry stages of decomposition were observed for the carcasses above and below the ground. The stages of decomposition were similar at both the depths. Time taken by all the carcasses in each stage of decomposition is represented in the Fig. 3.

Approximately 1800 insect specimens from Diptera, Coleoptera and Hymenoptera orders were collected from the carcasses T_{2022} and T_{2023} above the ground. While approximately 950 insect specimens were collected from the buried carcasses. Insects belonging to orders Diptera, Coleoptera, and Isopoda were common on the carcasses ($B_{40}\text{EF}_{2022}$ and $B_{80}\text{EF}_{2022}$) buried at 40 and 80 cm depths respectively. Species belonging to order Hemiptera were found visiting the buried carcass $B_{80}\text{EF}_{2022}$ at 80 cm depth. The decomposition stages and entomofauna associated with the carcasses above the ground and in graves can be described as follows (Tables 2 and 3):

During the exhumation of undisturbed carcass buried at 40 cm depth ($B_{40}\text{EO}_{2022}$), it was observed that there was more tissue left as compared to the disturbed one ($B_{40}\text{EF}_{2022}$). During the exhumation only adults of beetle *Saprinus sp.* and *Necrobia rufipes* were found. Amongst the dipterans, pupal cases of *Megaselia scalaris* were encountered. The $B_{80}\text{EO}_{2022}$ carcass buried at 80 cm depth was found to be in the early dry stage and tissue loss was lesser as compared to $B_{80}\text{EF}_{2022}$ carcass. Undisturbed carcasses ($B_{80}\text{EO}_{2022}$ & $B_{80}\text{EO}_{2023}$) were found devoid of any insect activity at this depth. The number of insect species from different orders is shown in the Table 4.

Discussion

A cadaver's decomposition in a buried environment is a multifaceted process and can be affected by several variables such as soil composition, temperature, moisture, and burial depth. A lesser temperature difference was recorded in the buried carcasses at 40 and 80 cm depths as compared to that on the surface. The depth of burial could be the reason behind this because the carcasses in graves were protected by the soil coverings and weren't exposed to direct heat. The rate of decomposition was also found to be slower for the carcasses ($B_{40}\text{EF}_{2022}$ and $B_{80}\text{EF}_{2022}$) buried at different depths as compared to the subaerially exposed carcass (T_{2022}). Similar findings

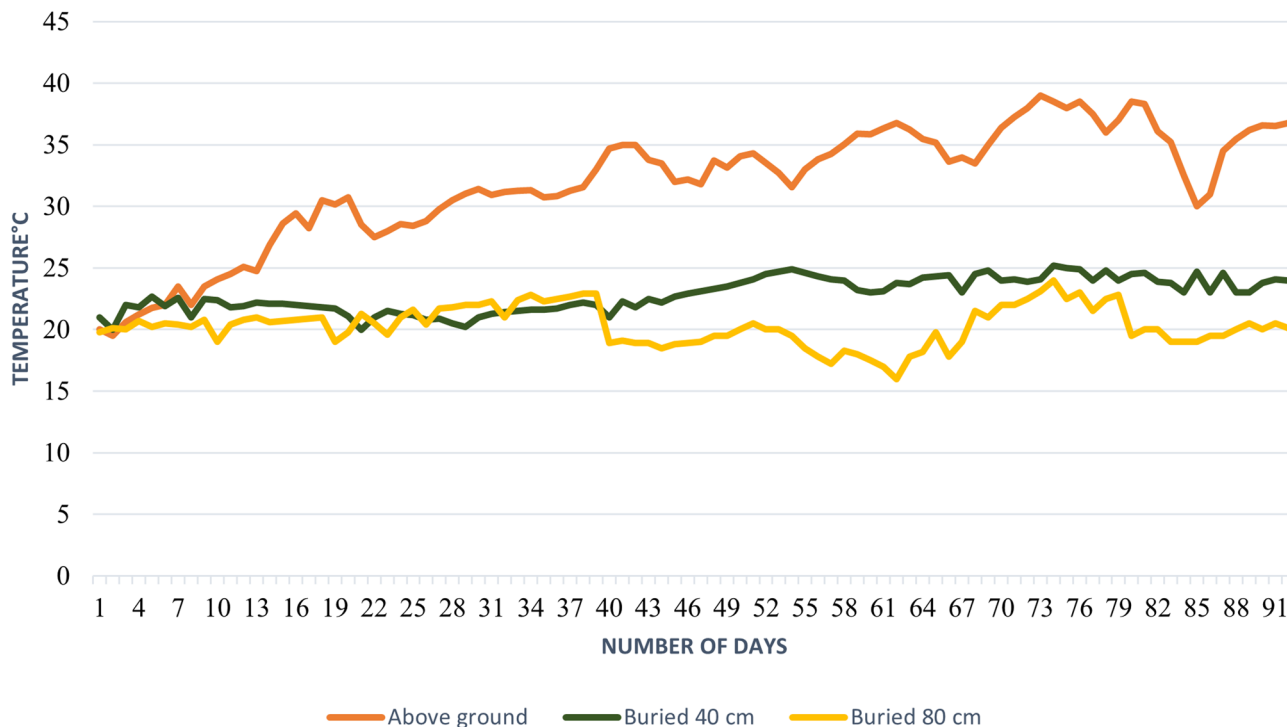


Fig. 1. Daily average temperature variations above and below the ground throughout the study period.

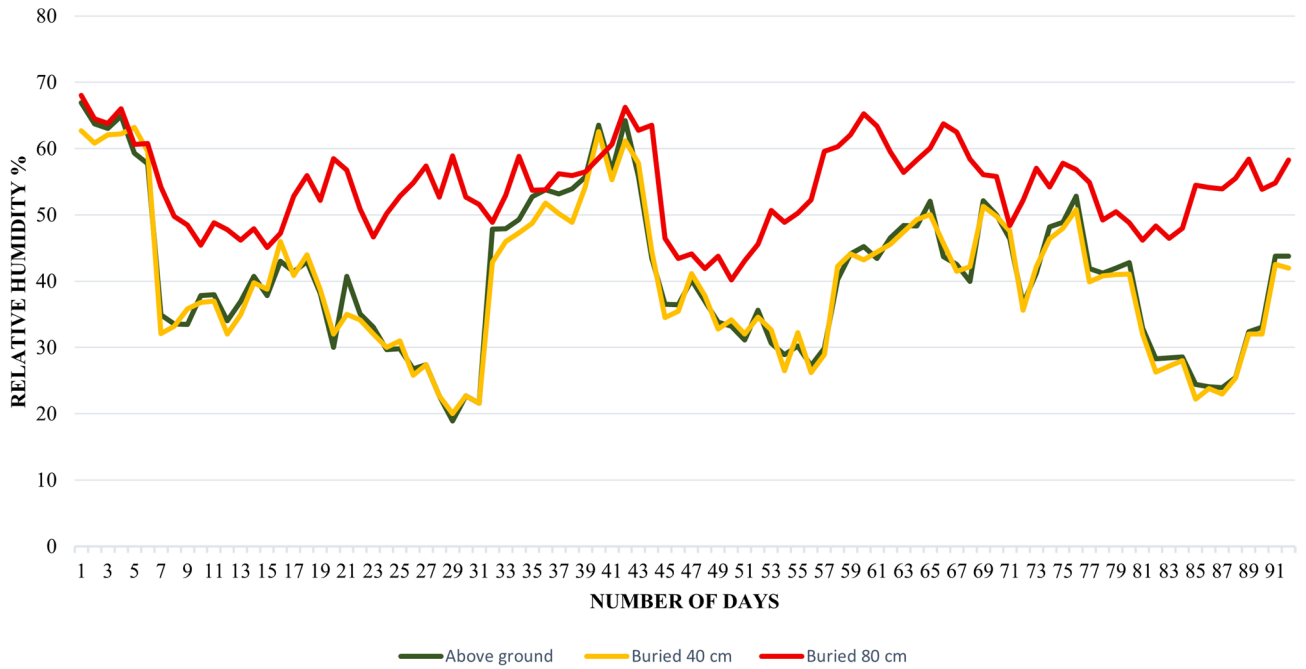


Fig. 2. Daily average humidity variations above and below the ground throughout the study period.

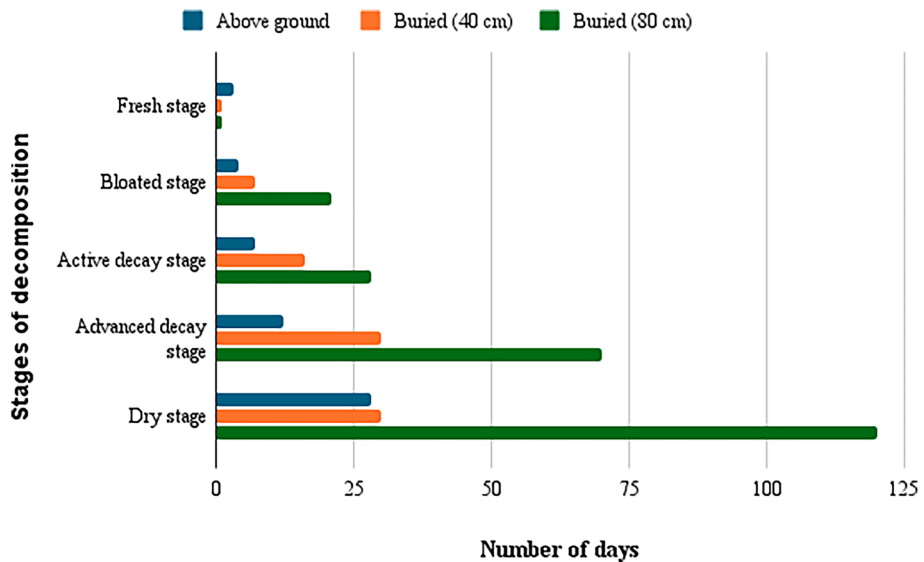


Fig. 3. Comparison of duration of stages of decomposition of carcasses placed above the ground and in the graves.

have also been observed in previous studies^{9,13,18,26–29} who noted that the decomposition gets prolonged due to reduced exposure to oxygen, temperature and access of insects to carcass.

It was interesting to observe that the rate of decomposition also varied at different burial depths. The decomposition rate was found to be much slower for the carcass ($B_{80}EF_{2022}$) buried at a depth of 80 cm as compared to the one buried at 40 cm depth ($B_{40}EF_{2022}$). The depth of burial is inversely proportional to the accessibility of the insects to the cadaver. Troutman et al.³⁰ pointed out that the decomposition of the buried cadaver can take up to 8 times longer than the cadaver placed above the ground, which was also proven in the present study for the carcass buried at 40 cm depth. The $B_{40}EF_{2022}$ carcass took up to 7 times longer to decompose than the T_{2022} carcass. While at the 80 cm depth, this duration was even longer. A study at 75 cm depth conducted by Werner et al.¹³ too has reported that the decay rate of buried carcass can be 55 to 83% lesser than the surface remains which was found to be similar to the present study at a depth of 80 cm whereby the decay rate of $B_{80}EF_{2022}$ was 90% lesser than the carcass (T_{2022}) on the terrestrial surface.

Stages of decomposition	Description	Insect activity
Fresh stage (0–3 days)	(a) Not any noticeable changes were observed	(a) No fly activity noted on day one till evening (b) MD visited the carcass (c) CM, CR and SH flies oviposited and larviposited on day 2. Their 1st instar larvae found feeding on carcass on day 3
Bloated stage (4–7 days)	(a) Bloating due to accumulation of gases produced due to anaerobic protein decomposition via microbial activity (b) Abdominal area turned green (c) Foul odor emanating from the carcass	(a) MD, CM, CR and SH still active on carcass. 2nd and 3rd instars of CM, CR and SH found feeding on the carcass on day 4 and 6 respectively (b) The larvae entered the pupal stage at the end of this stage
Active decay stage (8–14 days)	(a) Release of purge fluid, odor of decay intensified, and tissue started depleting	(a) MD, CM, CR, PC, SH and C found feeding on the carcass along with the emergence of first generation of CM, CR, and SH
Advanced decay stage (15–26 days)	(a) Soft tissues had been depleted (b) Only teeth, bones, cartilage, hair and some soft tissue left behind	(a) DM, NR and S found feeding on the carcass. (b) Larvae of the DM and NR were active beneath the carcass
Dry stage (27–54 days)	(a) Only dried skin, hair, teeth, cartilage and bones left behind	(a) Beetles dominated this stage, and no insect activity was noted after day 54

Table 2. Decomposition and insects associated with the pig carcasses on terrestrial surface. **Musca domestica* Linnaeus, 1758 (MD), *Sarcophaga haemorrhoidalis* Fallen, 1817 (SH), *Chrysomya megacephala* Fabricius, 1794 (CM), *Chrysomya rufifacies* Macquart, 1843 (CR), *Piophilha casei*, Linnaeus, 1758 (PC), *Dermestes maculatus* de Geer 1774 (DM), *Necrobia rufipes* de Geer 1775 (NR), *Saprinus* sp. Erichson, 1834 (S), and *Camponotus* sp. Fabricius (C).

Stages of decomposition	Description of decomposition stages		Insect activity	
	40 cm	80 cm	40 cm	80 cm
Fresh stage	This stage had passed off on the first exhumation on day 8	This stage had passed off on the first exhumation on day 8	–	–
Bloated stage	(a) $B_{40}EF_{2022}$ and $B_{40}EF_{2023}$ carcasses were in bloated stage when exhumed on 8th day of burial (b) Carcass had become fragile (c) Abdominal area appeared green (d) Odor was strong	(a) On day 8 carcass showed the signs of bloated stage at the abdominal area and in complete bloated stage on day 15 (b) Skin of abdominal area had become soft and easily rubbed off (c) Odor was strong during exhumation	(a) MS, SH and MD flies visited the carcass (b) 1st instar larvae of the MS and SH (feeding on head and abdominal region)	(a) Adults and 1st instar of MS were feeding on the head and abdominal region, in the cavities of eyes and underneath the limbs
Active decay stage	(a) On 15 it was found that carcass $B_{40}EF_{2022}$ entered this stage (b) Leaching of decomposition fluid (c) Hair started falling (d) Disarticulation of limb bones, hooves and cloven (e) Abdominal region deflated (f) In case of $B_{40}EF_{2022}$, white foam like material was observed beneath the carcass but this was not observed in the replicated study ($B_{40}EF_{2023}$)	(a) $B_{80}EF_{2022}$ and $B_{80}EF_{2023}$ carcasses were found in the active decay stage on day 29 and 35 respectively (b) Falling of hair and disarticulation of limb bones started (c) Abdominal region deflated	(a) MS, MD, SH, SF and NR and 3rd instars larvae of MS and SH found feeding on carcass (b) In addition, AC was found on the carcass ($B_{40}EF_{2023}$) in the replicate study	(a) Larvae of MS found to be at pupation (b) New 2nd and 3rd instars of MS (c) NR, SF and AC found visiting the carcass
Advanced Decay stage	(a) This stage appeared on day 30, soft tissues have been broken (b) Skin which was in contact with soil turned pink (c) Cheesy order (d) Discoloration of brown color in limbs (e) This stage of decomposition lasted for 30 days in the previous year and for 40 days in the replicated carcass ($B_{40}EF_{2023}$)	(a) On day 50 and 65 $B_{80}EF_{2022}$ and $B_{80}EF_{2023}$ carcasses were found to be in the advanced decay stage (b) Adipocere formation (c) Odor was cheesy in nature (d) Detachment of bones	(a) S and P species found feeding the carcass	(a) NR, SF, AC and OM found on and around the carcass
Dry stage	(a) $B_{40}EF_{2022}$ carcass entered in dry stage in thorax (60 days) and full body (90 days) (b) The replicated carcass ($B_{40}EF_{2023}$) entered the complete dry stage in 100 days	(a) Total dry stage appeared in 240 days (b) Cartilages, bones and limb detached, Skull was also skeletonized	(a) NR, H, P and A found on the carcass	(a) A, SF and OM found feeding the carcass

Table 3. Decomposition and insects associated with the pig carcasses buried at 40 and 80 cm depths. **Megaselia scalaris* Loew 1866 (MS), *Musca domestica* (MD), *Sarcophaga haemorrhoidalis* (SH), *Necrobia rufipes* (NR) *Saprinus* sp. (S), *Anthelephila caeruleipennis* La Ferte-Seneclere, 1847 (AC), Histeridae sp. (H), *Paederus* sp. (P), *Syntomus foveatus* Geoffroy, 1785 (SF), *Orius minutus* Linnaeus, 1758 (OM) and Armadillidae (A).

It was observed that frequent exhumation of a buried carcass significantly impacted the decomposition process and insect fauna. Each exhumation disturbs the burial environment, altering factors such as temperature, moisture levels and introduction to new insect species. The undisturbed carcass buried ($B_{40}EO_{2022}$) was found to retain more tissue mass as compared to the disturbed one ($B_{40}EF_{2022}$) during the exhumation which can be credited to the presence of diversity of insects on the disturbed one ($B_{40}EF_{2022}$). Although both carcasses were at the dry stage, a substantial amount of tissue mass remained underneath the dry skin in the $B_{40}EO_{2022}$ carcass owing to the presence of only two beetle species (*Saprinus* sp. and *Necrobia rufipes*) and one dipteran species (*Megaselia scalaris*). The replicated carcass $B_{40}EO_{2023}$ was also in the dry stage when exhumed and the tissue mass loss was almost same for both carcasses ($B_{40}EO_{2022}$ & $B_{40}EO_{2023}$). Bonacci et al.¹⁷ have also worked on

Insect order	Carcasses		
	Above ground	40 cm depth	80 cm depth
Diptera	5	3	1
Celeoptera	3	6	3
Hemiptera	0	0	1
Hymenoptera	1	0	0
Isopoda	0	1	1

Table 4. Number of insect species on the carcasses above the ground and in the buried environment.

the influence of frequent exhumation on the insect's arrival on the buried carcasses. Though they found that the buried carcasses were in adipocere condition when exhumed only once with no colonization by the insects, they too have reported that frequently exhumed carcass was in the advanced decay stage at that time with a lot of activity of the insects. The present study is in congruence to Bonacci et al.¹⁸ for undisturbed carcasses ($B_{80}EO_{2022}$ & $B_{80}EO_{2023}$) buried at a depth of 80 cm wherein no insect activity was found. Undisturbed carcasses ($B_{40}EO_{2022}$ & $B_{80}EO_{2022}$) at 40 cm and 80 cm depths also showed significant differences in decomposition rates. The decay rate was found to be slower at 80 cm depth as compared to the 40 cm depth. It was found that there was more tissue on the carcass $B_{80}EO_{2022}$ when it was exhumed only once as compared to the $B_{40}EO_{2022}$. These differences can be attributed to the depth of the burials. The forensic entomologists must carefully assess the burial site for signs of disturbance and consider these factors which might have altered the typical decomposition process. Frequent exhumation tends to lead to more rapid decomposition processes due to increased insect activity and variable microclimates (more exposure to the air). While undisturbed environments result in slower decomposition rate due to the lesser insect activity.

The succession pattern of entomofauna was the same for all the carcasses in both environments. Dipteran species were the early arrivers as well as the colonizers on the carcasses followed by the coleopterans. Variation in the arrival of insect fauna was observed above and below the ground in terms of the individual species. Blow flies (Calliphoridae) were the earliest visitors and colonizers followed by species belonging to Sarcophagidae and Muscidae families above the ground (T_{2022} and T_{2023}) and this has been reported in the previous studies in different seasons^{28,31–34}. According to Mabika et al.³⁵ species belonging to Calliphoridae were also the early colonizers followed by Sarcophagidae. *Musca domestica* and *Sarcophaga sp.* have also been reported previously^{28,36,37}. *Piophilha casei* species was also observed on the subaerially exposed carcasses (T_{2022} and T_{2023}) during the present study and have also been reported by Bonnaci et al.¹⁸ and Thakur and Kumari³⁶. *Sarcophaga haemorrhoidalis*, *P. casei*, and hister beetle have also been reported in India on the rabbit carcasses³⁸. *Dermestes maculatus* and *N. rufipes* species were early colonizers amongst the coleopteran species followed by the *Saprinus sp.* These beetle species have also been observed in previous works^{32,36,39}. As the decomposition process progresses, beetles are known to be of great forensic importance³¹. Adults and larvae of beetles are well recognized to consume dried tissue and remains⁴⁰. *Chrysomya megacephala*, *C. rufifacies*, *M. domestica*, *Dermestes maculatus*, *Necrobia rufipes*, Histeridae, and Formicidae have also been mentioned in a previous study³⁴.

Megaselia scalaris was found to be active on the buried carcasses ($B_{40}EF_{2022}$ and $B_{80}EF_{2022}$) at both depths in the current study. It was the only fly species which was able to access and reach the carcass ($B_{80}EF_{2022}$) buried at 80 cm depth during the present study. Due to its smaller size, *M. scalaris* is more easily able to make its way through the cracks in the soil in order to access the buried carcasses⁴¹. They prefer the darkness and narrow spaces and their ability to dig to various depths was confirmed in the present study because they were found at both the depths. *Megaselia scalaris* have also been reported in the previous studies at different depths^{9,17,18,29,42}. Similarly, Pastula and Meritt⁹ reported the colonization by this fly at both 30 and 60 cm depth. They also reported the colonization by flesh flies (*S. bullata*) at 30 cm depth and of *H. capensis* at both 30 and 60 cm depth. These two species were not found during the current study. In the present study *S. haemorrhoidalis* colonized the buried carcass ($B_{40}EF_{2022}$) along with the *M. scalaris*. *Megaselia scalaris* has also been reported in India on the buried goat carcass¹⁵. *Musca domestica* was found to be active on the buried carcass ($B_{40}EF_{2022}$) at 40 cm depth which has also been recorded on the corpses buried at different depths^{28,43}. Bourel et al.¹⁶ examined the coffins buried at 60 cm depth in Northern France. They found 3 species of Phoridae family, but they didn't report the *M. scalaris* at this depth in any of the exhumations. But in the present research, *M. scalaris* was the dominant fly at both the depths. This can be credited to the change in the environmental parameters in both the regions and forensic entomologists should also take into consideration the influencing factors of the particular area.

Necrobia rufipes (Cleridae) and *Syntomus foveatus* (Carabidae) arrived on the carcasses ($B_{40}EF_{2022}$ and $B_{80}EF_{2022}$) at both depths. *Necrobia rufipes* species has been reported by Pastula and Merritt⁹ and Bala and Kaur⁴⁴ at different depths and other species of Carabidae family have also been recorded in previous works^{18,44}. Bala and Kaur⁴⁴ only documented the presence of beetles on the buried pork liver and no flies were found on the porcine liver. On the contrary, during the present study the fly species were able to access and colonize the buried carcasses at both depths. In the present study, *Anthelephila caeruleipennis* were found at the 80 cm depth. Correa et al.²⁶ too reported the Anthicidae species at the 30 cm depth, but they considered it as an incidental insect because of their low abundance but in the present study these insects were higher in numbers at 80 cm depth and were found on three different sampling occasions.

In forensic investigations, understanding the process of carcass decomposition at different depths can greatly improve the accuracy of estimation of PMI. The decomposition rate is impacted by various factors, including temperature, and moisture which vary with depth. By examining these variations, forensic scientists can develop

more precise models to determine the time since death. This can be mainly critical in criminal cases where accurate PMI can impact the direction of investigations and the delivery of justice. The present study was the first one to study the effects of burial depths on the decomposition and associated entomofauna in a semi-arid region of India. The results of the study showed differences in the decomposition rates and entomofauna on carcasses above and below the ground, signifying their importance to investigate cases with buried bodies in a similar condition. With the identification of these species, their temporal arrival and colonization, forensic entomologists can take one step closer to estimating the PMI_{min} for buried cadavers. This study presents first-hand data on the arrival and colonization of the pig carcasses by the entomofauna in burial environment at two different depths. In addition to this, it also presents a comparison of the decomposition and insect fauna associated with the disturbed and undisturbed buried carcasses. The results point to the fact that the decomposition rate, insect arrival and colonization is greatly influenced by the frequent exhumation. Therefore, more research in this area is required to study the decomposition pattern and entomofauna associated with the undisturbed buried carcasses in real time.

Conclusion

The decomposition rate and insect fauna of carcasses exhibit significant differences in the burial environment and on the surface. The present study also concludes that environments significantly influenced the decomposition rate and insect fauna of carcasses. The carcasses decompose at the fastest rate on the surface due to direct exposure to environmental conditions which act as a driving force. Carcasses buried at a depth of 40 cm exhibited a slower decomposition rate compared to surface carcasses but decomposed more rapidly than those buried at 80 cm depth. The diversity and number of insect species associated with carcasses were found to be different at varying depths and on the surface. Surface carcasses attracted a wide range of insect species. Shallow-buried carcasses also attracted a variety of insects, although the diversity and abundance were lesser than those found on the surface. Deep-buried carcasses exhibited the least diversity and number of insect species, primarily involving the small-size soil-dwelling insects capable of penetrating the burial depth. *Megaselia scalaris* was the dominating species at both the depths and can play an important role in the estimation of post burial interval along with the help of beetle species found at these depths. These findings underscore the critical role of environmental exposure and burial depth in influencing both the decomposition process and the succession of insect fauna. This knowledge has practical applications in forensic science for estimating post-burial intervals and can enhance our understanding of ecological processes related to carrion decomposition in different environments. The absence of facilities to observe the decomposition and insect assemblages of the undisturbed buried carcasses remains a lacuna of the present study which can be attributed to the expenses involved. Nevertheless, this is also an opportunity for further research in this area.

Data availability

All data generated or analyzed during this study is included in this manuscript.

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References

- Tibbett, M. & Carter, D. O. Research in forensic taphonomy: a soil-based perspective. In *Criminal and Environmental Soil Forensics*. 317–331 (Springer Netherlands, 2009).
- Dirkmaat, D. *A Companion To Forensic Anthropology* (Wiley, 2012).
- Rai, J. K., Pickles, B. J. & Perotti, M. A. The impact of the decomposition process of shallow graves on soil mite abundance. *J. Forensic Sci.* **67**(2), 605–618 (2022).
- Pittner, S. et al. A field study to evaluate PMI estimation methods for advanced decomposition stages. *Int. J. Legal Med.* **134**, 1361–1373 (2020).
- Harvey, M. L., Gasz, N. E. & Voss, S. C. Entomology-based methods for estimation of postmortem interval. *Res. Rep. Forensic Med. Sci.* **6**, 1–9 (2016).
- Amendt, J. et al. Best practice in forensic entomology—standards and guidelines. *Int. J. Legal Med.* **121**, 90–104. <https://doi.org/10.1007/s00414-006-0086-x> (2007).
- Reibe, S. & Madea, B. Use of *Megaselia scalaris* (Diptera: Phoridae) for post-mortem interval estimation indoors. *Parasitol. Res.* **106**, 637–640. <https://doi.org/10.1007/s00436-009-1713-5> (2010).
- Wang, M. et al. Forensic entomology application in China: four case reports. *J. Forensic Legal Med.* **63**, 40–47 (2019).
- Pastula, E. C. & Merritt, R. W. Insect arrival pattern and succession on buried carrion in Michigan. *J. Med. Entomol.* **50**(2), 432–439. <https://doi.org/10.1603/ME12138> (2013).
- Kyerematen, R. A., Boateng, B. A., Haruna, M. & Eziah, V. Y. Decomposition and insect succession pattern of exposed domestic pig (*Sus scrofa* L.) carrion. *ARPJ. Agricultural Biol. Sci.* **8**(11), 756–765 (2013).
- Iancu, L., Carter, D. O., Junkins, E. N. & Purcarea, C. Using bacterial and necrophagous insect dynamics for post-mortem interval estimation during cold season: novel case study in Romania. *Forensic Sci. Int.* **254**, 106–117. <https://doi.org/10.1016/j.forsciint.2015.07.024> (2015).
- AL-Mekhlafi, F. A., Al-Zahrani, O., Al-Qahtni, A. H. & Al-Khalifal, M. S. Decomposition of buried rabbits and pattern succession of insect arrival on buried carcasses. *Int. J. Trop. Insect Sci.* **44**, 1037–1044. <https://doi.org/10.1007/s42690-024-01203-x> (2024).
- Marais-Werner, A., Myburgh, J., Becker, P. J. & Steyn, M. A comparison between decomposition rates of buried and surface remains in a temperate region of South Africa. *Int. J. Legal Med.* **132**, 301–309 (2018).
- Singh, R., Sharma, S. & Sharma, A. Determination of post-burial interval using entomology: a review. *J. Forensic Legal Med.* **42**, 37–40 (2016).
- Sharif, S., Singh, C. P., Athar, B., Khan, M. K. & Qamar, A. Forensic and ecological significance of necrophagous insects: insights from animal carcasses, human cadavers, and myiasis patients. *Legal Med.* **71**, 102544. <https://doi.org/10.1016/j.legalmed.2024.102544> (2024).
- Bourel, B., Tournel, G., Hédouin, V. & Gosset, D. Entomofauna of buried bodies in Northern France. *Int. J. Legal Med.* **118**, 215–220. <https://doi.org/10.1007/s00414-004-0449-0> (2004).

17. Mariani, R., García-Mancuso, R., Varela, G. L. & Kierbel, I. New records of forensic entomofauna in legally buried and exhumed human infants remains in Buenos aires, Argentina. *J. Forensic Legal Med.* **52**, 215–220. <https://doi.org/10.1016/j.jflm.2017.09.012> (2017).
18. Bonacci, T. et al. Investigations on arthropods associated with decay stages of buried animals in Italy. *Insects* **12**(4), 311. <https://doi.org/10.3390/insects12040311> (2021).
19. Zou, T. L., Feng, D. X., Huang, G. Y., Sun, D. P. & Dai, S. T. Species composition and succession of necrophagous insects on small buried baits in China. *J. Med. Entomol.* **59**(4), 1182–1190 (2022).
20. Sharif, S. & Qamar, A. Insect faunal succession on buried goat carcass in Aligarh region of Uttar Pradesh, India, with implications in forensic entomology. *Egypt. J. Forensic Sci.* **11**, 1–8 (2021).
21. Wescott, D. J. Recent advances in forensic anthropology: decomposition research. *Forensic Sci. Res.* **3**(4), 278–293 (2018).
22. Dias, R. K. Taxonomy key for the subfamilies of worker ants (Family: Formicidae) in Sri Lanka and some information on anetureus simoni Emery in Ratnapura. (2004).
23. Couri, M. S., De Carvalho, C. J. & Pont, A. C. Taxonomy of the muscidae (Diptera) of Namibia: a key to genera, diagnoses, new records and description of a new species. *Afr. Invertebrates.* **53**(1), 47–67 (2012).
24. Sawaby, R. F., Hamouly, H. E. & Ela, A. E. Taxonomic study of the main families of Egyptian coleoptera with forensic importance. *Life Sci. J.* **13**(4), 39–53. <https://doi.org/10.7537/marslsj13041605> (2016).
25. Sawaby, R. F., Hamouly, H. E. & Ela, A. E. Diagnosis and keys of the main dipterous families and species collected from rabbit and Guinea pig carcasses in Cairo, Egypt. *J. Basic. Appl. Zool.* **79**, 1–14. <https://doi.org/10.1186/s41936-018-0018-6> (2018).
26. Corrêa, R. C., Almeida, L. M. & Moura, M. O. Coleoptera associated with buried carrion: potential forensic importance and seasonal composition. *J. Med. Entomol.* **51**(5), 1057–1066. <https://doi.org/10.1603/ME13166> (2014).
27. Al-Mekhlafi, F. A. Decomposition process for buried rat (*Rattus norvegicus*, Berkenhout 1769) carcasses in Riyadh city, Saudi arabia: A preliminary study. *Saudi J. Bio Sci.* **28**(7), 3745–3750. <https://doi.org/10.1016/j.sjbs.2021.03.075> (2021).
28. Al-Zahrani, O., Al-Khalifa, M. S., Al-Qahtni, A. H. & Al-Mekhlafi, F. A. Decomposition and dipteran succession on buried rabbits carcasses. *Saudi J. Bio Sci.* **30**(11), 103822. <https://doi.org/10.1016/j.sjbs.2023.103822> (2023).
29. Botham, J. L. *Decomposition and Arthropod Succession on Buried Remains during Winter and Summer in Central South Africa: Forensic Implications and Predictive Analyses* (University of the Free State, 2016).
30. Troutman, L., Moffat, C. & Simmons, T. A preliminary examination of differential decomposition patterns in mass graves. *J. Forensic Sci.* **59**(3), 621–626 (2014).
31. Bharti, M. & Singh, D. Insect faunal succession on decaying rabbit carcasses in Punjab, India. *J. Forensic Sci.* **48**(5), JFS2001358. <https://doi.org/10.1520/JFS2001358> (2003).
32. Wang, J., Li, Z., Chen, Y., Chen, Q. & Yin, X. The succession and development of insects on pig carcasses and their significances in estimating PMI in South China. *Forensic Sci. Int.* **179**(1), 11–18. <https://doi.org/10.1016/j.forsciint.2008.04.014> (2008).
33. Voss, S. C., Spafford, H. & Dadour, I. R. Annual and seasonal patterns of insect succession on decomposing remains at two locations in Western Australia. *Forensic Sci. Int.* **193**(1–3), 26–36. <https://doi.org/10.1016/j.forsciint.2009.08.014> (2009).
34. Wang, Y. et al. Insect succession on remains of human and animals in Shenzhen, China. *Forensic Sci. Int.* **271**, 75–86. <https://doi.org/10.1016/j.forsciint.2016.12.032> (2017).
35. Mabika, N., Masendu, R. & Mawera, G. An initial study of insect succession on decomposing rabbit carcasses in Harare, Zimbabwe. *Asian Pac. J. Trop. Biomed.* **4**(7), 561–565. <https://doi.org/10.12980/APJTB.4.2014C1031> (2014).
36. Thakur, J. & Kumari, U. Studies on incidence and succession of forensically important insects associated with decomposition of goat flesh. *J. Entomol. Zool. Stud.* **7**(4), 1427–1431 (2019).
37. Bernhardt, V., Bálint, M., Verhoff, M. A. & Amendt, J. Species diversity and tissue specific dispersal of necrophagous diptera on human bodies. *Forensic Sci. Med. Pathol.* **14**(1), 76–84. <https://doi.org/10.1007/s12024-018-9947-0> (2018).
38. Sharma, S., Sharma, A. & Singh, R. Effect of seasonal and altitudinal variation on insect succession and rabbit carcass decomposition in West Himalayan region of India. *Anil Aggrawals Int. J. Forensic Med. Toxicol.* **23**, (2022).
39. Singh, N. & Bala, M. Succession study on forensically important coleoptera from india: a preliminary study and its forensic implications. *Egypt. J. Forensic Sci.* **9**, 1–8. <https://doi.org/10.1186/s41935-019-0168-9> (2019).
40. Schroeder, H., Klotzbach, H., Oesterhelweg, L. & Püschel, K. Larder beetles (Coleoptera, Dermestidae) as an accelerating factor for decomposition of a human corpse. *Forensic Sci. Int.* **127**(3), 231–236. [https://doi.org/10.1016/S0379-0738\(02\)00131-7](https://doi.org/10.1016/S0379-0738(02)00131-7) (2002).
41. Manlove, J. D. & Disney, R. H. L. The use of *Megaselia abdita* (Diptera: Phoridae) in forensic entomology. *Forensic Sci. Int.* **175**(1), 83–84. <https://doi.org/10.1016/j.forsciint.2007.08.001> (2008).
42. Mariani, R., García-Mancuso, R., Varela, G. L. & Inda, A. M. Entomofauna of a buried body: study of the exhumation of a human cadaver in Buenos Aires, Argentina. *Forensic Sci. Int.* **237**, 19–26 (2014).
43. Kekillioglu, A. & Başar, M. Research on the ecological success role of the muscidae (Insecta: diptera) species. *Eurasian J. Sci. Eng. Tech.* **2**(1), 36–42 (2021).
44. Bala, M. & Kaur, P. Insect faunal succession on buried piece of pork in the state of Punjab (India): A preliminary study. *J. Forensic Res.* **5**(6), 1–4 (2014).

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Author contributions

All the authors have contributed significantly and are in agreement with the content of the manuscript. Their roles were as follows- P.S.- Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing—original draft. S.S.- Conceptualization, Methodology, Resources, Supervision, Validation, writing – review and editing. R.T.- Data curation, Formal analysis. N.V.- Data curation, Formal analysis. J.A.- Editing.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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