Determination of post-burial interval using entomology: A review

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ABSTRACT

Insects and other arthropods are used in different matters pertinent to the criminal justice system as they play very important role in the decomposition of cadavers. They are used as evidence in a criminal investigation to determine post mortem interval (PMI). Various researches and review articles are available on forensic entomology to determine PMI in the terrestrial environment but very less work has been reported in context to buried bodies. Burring the carcass, is one of the methods used by criminals to conceal the crime. So, to drive the attention of researchers toward this growing field and to help various investigating agencies, the present paper reviews the studies done on determination of post-burial interval (PBI), its importance and future prospective.

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1. Introduction

Forensic science is a multifaceted branch, and Forensic Entomology is one of the important branches of forensic science, which primarily used for the estimation of the time since death or PMI by studying stages of decomposition, pattern of arthropod succession or estimating the age of the immature stages of the arthropods present at the scene. The data of forensically important arthropods for terrestrial habitat is very well established and also utilized worldwide for helping the investigating agencies one way or other. But, there are still some aspects of forensic entomology which are untouched or on which very little work has been reported, and post-burial interval determination is one of such aspect of forensic entomology. Thus, it is the need of time to make an effort and develop more expertise in this particular field.

As bodies of the victims are sometimes left where they are killed but, there is usually an attempt to conceal or dispose of the evidence. These attempts range from hiding the body behind the nearest hedge to placing it in water stream or acid bath. A relatively common method of concealment is burial, it is a religious act of placing a dead person or animal, into the ground. A pit or trench was dug and the deceased was placed in it, and covered. Burial is one of the methods used for religious practices in old time. There are some religious reasons of burial, but along with these reasons, there may be a criminal intent to burial. Thus, we have to look into both pros and cons of the burial. But, depending upon the weight and size of the body an effort is required to dig to any depth. Thus, in most of the criminal cases victims are buried in shallow clandestine graves and in fact, after 72 h, forensic entomology is usually the most accurate and often the only method for determining PMI. There are several studies on the colonization of dead bodies by insects but many of these relate to bodies placed on the surface of the ground rather than underneath it. Thus, it is important to understand the factors that affect insect colonization because by studying those one can predict when, and sometimes where a person died.

When an organism dies, there is a sequential colonization by arthropods (mainly Diptera and Coleoptera), that originates a predictable ecological succession directly related to the various stages of decomposition. Using these arthropods we can; determine time since death, medico-legal questions regarding the surroundings and hygiene of the scene, physical abuse or neglect if any, determination of poisons or drugs, food contamination etc. There are many factors that alter the pattern such as, environmental changes, physical barriers (water, plastic bags etc.), or cultural intervention related to funerary practices. The burial environment can also alter the rate of decomposition, and subsequently impact the estimation of time since death. Bodies buried in soil demonstrate a slower rate of decomposition than bodies placed over a soil surface in the same environment. The environment in which a body is buried is generally defined by the chemical, biological and geological conditions of the location. Factors such as, the depth of the burial, the presence or absence of a coffin, the physical composition of the body, clothing type and the physical conditions
of the soil, such as texture, pH, moisture, temperature and oxygen content can affect decomposition within a burial site.\textsuperscript{11}

2. Post-burial interval

Various researchers have contributed to the field of forensic entomology, by keeping in view various aspects and factors that affect the forensic entomology. The first study on buried cadavers was reported in 1985. This study utilized six un-embalmed human cadavers, buried separately in unlined trenches of various depths and allowed to decompose naturally. It took about a year to complete this study, and during this period of burial, sampling was done daily from the air and soil, and cadaver temperature at each burial site was also recorded. After each specified burial period the cadavers were dug out and examined for degree of decomposition along with the changes in the pH of the soil, surface vegetation, and carrion insect activity. From this study it was found, that the decomposition rate of buried cadavers is highly dependent on the depth of burial and environmental temperatures, depth at which the cadaver was buried also directly affected the degree of soil and vegetation changes as well as access to carrion insects.\textsuperscript{12} As this was the first study conducted on burial cadaver and some of the important factors such as depth of burial, pH of soil, temperature etc. which affects the carrion decomposition and insect succession were studied. So, moving further in the forward directions, to determine post-burial interval, in 1999 Vanlaerhoven and Anderson, conducted an experiment using pig carcass to establish a database of insect succession on buried carrion in two biogeoclimatic zones of British Columbia. The pig carcasses were buried shortly after death in the Coastal Western Hemlock and Subboreal Spruce bio-geoclimatic zones of British Columbia. Buried pigs exhibited a distinct pattern of succession from that which occurred on above ground carrion and the species composition and time of colonization differed between the two zones. Thus, it is concluded that, ideally a database of insect succession on buried carrion should be established for each major biogeoclimatic zone and soil temperature should be used to determine developmental rates of insects for determination of the postmortem interval.\textsuperscript{13}

Based on literature following table is formed in which post burial insect fauna of forensically importance is listed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Calliphora vicina</td>
<td>Calliphoridae</td>
<td>Gunn and Bird (2011)</td>
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<td>Muscina stabulans</td>
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<td>Lucilia sericata</td>
<td>Calliphoridae</td>
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<td>Muscina prolapsa</td>
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<td>Muscina levida</td>
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<td>Calliphora vomitoria</td>
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<td>Muscina stabulans</td>
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<td>Calliphora vicina</td>
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<td>Tinea pellionella</td>
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<td>Tinea bisselliella</td>
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<td>Fannia scalaris</td>
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<td>Mariani et al. (2014)</td>
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<td>Ophyra aeneascens</td>
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<td>Megaselia scalaris</td>
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<td>Tineola bisselliella</td>
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<td>Eumacrorychnia persolla</td>
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<tr>
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<td>Lord et al. (1992)</td>
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<td>Calliphora vicina</td>
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<tr>
<td>Conicerca Tibalis</td>
<td>Phoridae</td>
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2.1. Importance of blowflies in cases of burial

Blowflies are the first visitors on the carcass above ground as reported in the literature. Some researchers consider that even a thin covering of soil will prevent a body being colonized by blowflies because the female flies require physical contact with a suitable larval food source before they will lay their eggs. Despite this, there are several reports of adult blowflies being attracted to the site of a buried body by the smell emanating from the ground. For example, Rodriguez and Bass (1985) observed that, the adult blowflies laying eggs in the soil above remains buried at 30 cm deep and also attempting to reach them by crawling through cracks and crevices in the soil.\textsuperscript{12} Similarly, VanLaerhoven and Anderson (1999) observed Calliphora vicina laying eggs in the soil above remains buried at a depth of 30 cm; although that does not necessarily mean that the larvae subsequently reached the corpse.\textsuperscript{13} Thus, there is some uncertainty about the ability of blowflies to exploit buried remains and furthermore it is not known how burial of already flyblown (fly infested) remains affects the hatching of blowfly eggs and the subsequent development of the larvae. This is an important consideration because it could help determine how long the body had been buried.\textsuperscript{3}

Keeping this aspect in view, a research on the ability of the blowflies such as Calliphora vomitoria, C. vicina and Lucilia sericata to colonize pig liver baits buried in loose soil was conducted. The experiment included following parameters:

i. Ability of flies to exploit buried remains: It has been found that, blowfly C. vicina dominated the above ground bait fauna and was also found in large numbers on the buried liver samples but not on buried liver when supplemented by blood. Muscid flies were present on all the buried liver samples and M. stabulans and M. prolapsa were especially abundant when the liver was supplemented with blood.

ii. Ability of fly larvae to develop on buried remains: In both species C. vomitoria and L. sericata, the eggs were able to hatch underground and the larvae developed through to adult. The rate of development was same as that on surface bait. In M. stabulans and M. prolapsa, the adult flies laid their eggs on the soil surface and then the larvae crawled down to the bait. When the bait was on the surface of the soil, the flies laid their eggs in the soil around the bait, rather than on the bait.

iii. The influence of burial on pupariation behaviour: In C. vomitoria and L. sericata, the post-feeding 3rd instar larvae placed on the surface rapidly burrowed into the soil and some of them reached to the base (soil depth 25 cm) within 15 min. The majority of L. sericata formed puparia in the top (2 cm of soil) and C. vomitoria, developed into puparia over a wider range of depths, most were found at the top (8 cm). L. sericata puparia were often found embedded within clumps of hard soil or in fragments of vegetation. On the soil surface neither of the species larvae develops into puparia. In M. stabulans and M. prolapsa, the majority of larvae developing on surface bait formed puparia at the top (4 cm) of the soil. However, the overall distribution of M. stabulans puparia was significantly broader than that of M. prolapsa. There was no difference between the pupariation depths distributions of the two species when the larvae were developing on baits buried at 20 cm.

iv. Burrowing capabilities of adult flies: All the species of C. vomitoria and L. sericata, emerged from buried puparia that reached the surface regardless of the depth at which they were buried and those, did not reach the surface did not emerge from their puparium.

From experiment, it has been found that, the C. vomitoria colonized the baits buried at 5 cm but not deeper, while C. vicina and L.
sericata colonized the remains at 10 cm but not at 20 cm. It is concluded that, the baits were colonized by larvae hatched from eggs laid on the surface of the soil. Both C. vomitoria and L. sericata were able to develop from eggs through to adulthood on baits that were infested before being buried and the rate of larval development and pupa formation is very much similar in depth to that on the soil surface. And it is also observed that the muscid flies, Muscina stabulans colonized baits buried at 10 cm but Muscina prolapsa only colonized those buried at 5 cm and when presented with baits on the soil surface their larvae tend to remain in the soil beneath the baits. The adult flies were instantly attracted to feed on fresh blood and laid eggs in the soil above buried baits within 30 min but adult muscid flies did not attempt to burrow into the soil. Thus this information can be useful in determining whether a body was stored above ground before being buried. Application of this information can contribute to a more accurate estimation of time since death of a buried corpse and may aid in the location of such corpses.3

2.2. Factors affecting PBI determination

a. Seasonal variations

Along with the depth, temperature, vegetation, pH of the soil, seasonal variation etc. is also important factors to be studied. So, in 2014, Mariani et al. conducted a study for autumn season, and focused on insects and other arthropods sampled from an infant skeleton belonging to ‘Prof. Dr. Romulo Lambre’ skeletal collection. The skeletons were buried in soil inside a wooden coffin in a grave 40 cm deep, in autumn, and stored in the cemetery deposit after exhumation. The fauna from wrappings, clothes, bones and soil samples, were collected and identified at different taxonomic levels depending on the stage of conservation. The dominant taxon was of muscid fly (Ophyra aenesens), represented by a considerable amount of empty puparia and some fragmented adults, heads, abdominal tergites and legs. These remains were found on wrappings, the diaper, clothes and in bone cavities such as in a tooth socket and one of the ear canals. Other fly remains collected were of the family Sarcophagidae, M. stabulans (Muscidae), Fannia canicularis (Fanniidae), Megaselia scalaris (Phoridae), Lepidopteran moth Tineola bisselliella (Tineidae), some of the beetles such as Tenebrionidae, Dermestidae, Staphylinidae (Atheta sp.) and fragments of adults of Carphophilus sp. (Nitidulidae). It was also noted that, some puparia were present within other puparia, specifically in some O. aenesens puparia which were found within puparia of the same species, and some puparia of M. stabulans and M. scalaris were found inside puparia of M. stabulans. This finding suggests that flies bred on the corpse for several generations. It was found that, there is some relationship between the identified taxa and the moving of the corpse, from the burial in context to the cemetery deposit, and a hypothetical colonization sequence after death was also created using the above findings. It has been concluded based on study that the application of entomological data to anthropological research can provide valuable information for the interpretation of taphonomic processes and burial contexts.10

b. Habitat variation

The nature of the surrounding i.e. by varying the habitat (i.e. dried or moistened) Szpila et al. (2010) reported the first breeding records of miltogrammine fleshflies in buried carrion. The experiments with buried animal models were continued from 2001 to 2005 and every year one pig weighing approximately 23 kg was used as model. From the experiment it was observed that, the first instars of Eumacronychia persolla (Diptera: Sarcophagidae) and Phylloletes pictipennis (Diptera: Sarcophagidae) were able to penetrate dry, loose soil and reach deeply buried animal remains, an ability which is unique to necrophagous Calyptratae. P. pictipennis successfully completed development up to depth of 5 cm and 33 cm layers of dry Sand. It has been concluded that Calyptratae have broad geographical distribution, fast location and colonization of carrion, complete development on buried food resources and easy identification which make these species useful forensic indicators in buried bodies in dry habitats.14

c. Effect of physical barriers (e.g. suitcase)

In order to keep pace with modern trends of crimes, it became imperative to develop smart tools and techniques and even for detection of bodies concealed in bags. Accordingly, there is clairion call to study the accessibility of bodies in suitcases or any other materials like polythene bags etc. Bhadra et al. in 2014, made an attempt to study the factors affecting accessibility to blowflies of bodies disposed in suitcases and an experiment by incorporating different zips (toothed and coil) of various gauges (4–6 mm) above a chicken liver bait was conducted. The C. vomitoria and C. vicina were observed to be attracted and oviposited, through these zips, both under laboratory and field conditions. The process of egg laying was observed significantly more frequent and with greater numbers of eggs when zips were in contact with the bait than when they were placed approximately 6 cm above the bait and in the absence of bait, adult females could be stimulated to lay eggs on moistened zips, although the presence of blood accelerated egg laying compared to water alone and no eggs were laid on dry zips in the absence of bait. 89% of first instar larvae were able to colonize the bait below the zips by passing through gaps between the teeth. A preliminary field studies were also conducted using suitcases baited with a pig’s head and found that there was a delay of 1–3 days in oviposition when compared to laboratory conditions. This information has practical value in explaining the presence of larvae on enclosed bodies in suitcases and will help forensic entomologists estimate a more accurate minimum time since death.15

d. Effect of chemicals (such as lime)

As we live in modern world where chemicals like lime etc. are easily available and sometime criminals try to use such chemicals to conceal the crime. Such casework was reported by Schotsmans et al. (2012) in Belgium involving the search for human remains buried with lime. It was demonstrated that, it needs more detailed understanding of the effect of different types of lime on cadaver decomposition and its micro-environment. Thus keeping in mind six pigs were subjected as body analogues in field experiments. The pigs were buried without lime, with hydrated lime i.e. Ca(OH)2 and with quicklime i.e. CaO in shallow graves in sandy loam soil. After 6 months of burial carcasses were recovered and it is observed that hydrated lime and quicklime both delayed the decay of the carcass during the first 6 months. It has been concluded that, the study has implications for the investigation of clandestine burials and for a better understanding of archaeological plaster burials. Knowledge of the effects of lime on decomposition processes also has bearing on practices involving burial of animal carcasses and potentially the management of mass graves and mass disasters by humanitarian organizations.16

3. Case studies

Along with these research experiments some researchers have been trying to apply the experimental findings on real field cases so as to find the post-burial interval by utilizing forensic entomology. A case of badly decomposed body of a woman recovered from a shallow
grave located beneath a house in rural South Carolina, USA was recovered and from that 2 fly species (immature) were collected. One of these, *Synthesomyia nudiseta*, was a new record for the state and the other, *C. vicina*, is a common inhabitant of carrion worldwide. The immature flies were reared and subsequent degree day was calculated for these flies, when viewed in the context of local environmental conditions, allowed an accurate estimate of the approximate date of the victim’s death, the date was also confirmed subsequently by the confession of the assailant. The emphasis was given on the usefulness of entomological evidence in estimating time of death, particularly in cases where the postmortem interval is prolonged and the value of other methods is limited.17

In similar way Martin et al. (2011) reported about “coffin fly” *Conicera tibialis* (Order: Diptera, Family: Phoridae), well known for its frequent occurrence on buried corpses even in some cases after postmortem intervals of 35 years. The study was focused on the presence of a large amount of individuals of *C. tibialis* inside the coffin of a buried human corpse exhumed 18 years after death in central Spain. It has been observed that, the adults, newly emerged and empty puparia in connection with the remains and concluded such postmortem interval is significantly longer than previously known for this species and raises the question on the current state of knowledge about the use of insects for estimating the postmortem interval in old, buried remains.18

Apart from these case studies, a case of two young children of 11 and 13 years age missing on 5 June 2006 (06:30 p.m.) from a small town of Southern Italy was reported by Introna et al. (2011). Police and various other persons of community tried to find them safe however, every effort was in vain, and the search went on for more than 1 year. During the search, the investigators collected enough evidence against the father, who was arrested 17 months after the children’s disappearance. He was indicted for kidnapping, homicide and concealment of the two bodies. He never confessed to the crimes and claimed to be innocent. Three months after the conviction, a fireman found the two corpses in a subterranean, dry cistern next to a well over 20 m deep. The bodies were well preserved, almost mumified, with only few body-parts skeletonized. Based on dental records, they were identified as those of the two children, who had gone missing 1.5 years before. Signs of a very low insect activity were present, reasonably consistent with rapid skin dehydration. A long post-mortem interval (PMI) of approximately 20 months was estimated, mainly from the pattern of insect succession. Based on such evidence, the father was finally released from prison and exonerated from previous indictment of homicide.19

### 4. Conclusion and future prospect

Based on literature it can be concluded that, the field of Forensic entomology specially the Forensic burial entomology needs more emphasis. As Forensic entomology plays a vital role in determination of PMI and thus, we cannot leave such an important perspective. We know that, burring is one of the methods used for religious practice after death. But, it can also be used with criminal intent to conceal the crime and such concealment might delay detection of the carcass by people or investigating agencies, which will leads to difficulties in determination of PBL. Thus, the aspect of forensic burial entomology should be looked into so as to determine PBI and the research should be conducted in this particular direction of the field. The database of forensically important insect should be maintained for different geographical locations, with variation in factors such as; temperature, altitude, habitat, sunshine, indoor—outdoor etc. that affect entomology.

As every field has it’s applications as well as limitations, similarly forensic burial entomology has applications like using forensic burial entomology we can determine;

- PBI at variable depths, movement of the carcass etc.
- But this field has some limitations such as; very less work has been reported on materials or factors that can prevent access to the carcass worldwide and no work has been reported on the effect of depth more than 40 cm, the carcass buried in snow or deserts, the determination of poison taken, drug abused, physical neglect if any etc.

Thus, this field needs more exploration further, and researcher should consider various factors and must develop a global database.

### Conflict of interest

We all declare that there is no Conflict of Interest and this article is neither sent nor published in any other journal.

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