Brielle Cintron

Dr. Windfelder

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Forensic Anthropology and the environment: using soil, insects and plant material to determine postmortem interval

**Introduction**

 Estimating postmortem interval (PMI) is crucial to any forensic investigation. Not only does it give a timetable for a death, it can also help exclude possible victims and perpetrators. Two ways a forensic anthropologist may estimate PMI is through either antemortem or postmortem changes. Antemortem changes refers to any physiological or pathological conditions which are identified on the remains but were present prior to death. Postmortem changes are associated with any alterations to the corpse after death (Madea 2016). This paper will focus solely on using postmortem changes to estimate PMI. Postmortem lividity can be used early on after death and begins within twenty to thirty minutes after death. Lividity can be visually seen as pink patches that will change to dark pink or blue as time progresses. Pressure relieves lividity and can disappear with only thumb pressure early on. As PMI increases, so does the pressure needed to cause lividity to cease. However, after a certain amount of time (8-12 hours), lividity will no longer disappear upon pressure (Madea 2016).

Three additional early PMI estimation methods used are testing algor, rigor and livor mortis. Algor mortis is based on the cooling of the body. Once the temperature of the body is taken one must subtract that temperature from 98.6°F. For every 1.5°F drop it can be concluded that one hour has passed. This is based on the idea that a body loses 1.5 ºF per hour after death. Livor mortis is the pooling of blood in areas of the corpse closest to the ground or point of pressure. This stage begins within 1 hour and ceases between 9-12 hours after death. Rigor mortis is characterized by the stiffening of the body, starting 2-6 hours after death and continues developing over the first 12 hours (Goff 2009). Usually rigor mortis ends between 24-48 hours. After the 24-hour time period, the algor, livor and rigor mortis lose accuracy and are unusable once tissue has begun to decompose (Byers 2017).

Decomposition commences when tissue begins to break down. The processes of autolysis and putrefaction are partially responsible for decomposition. Autolysis takes effect quickly after death, usually within minutes (Zhou and Byard 2011). Digestive fluids that exist in the intestinal tract of a human begin to digest bodily tissues during the process of autolysis (Byers 2017). Putrefaction, or rotting, starts at a late stage of decomposition. There are five main stages of decomposition: fresh, bloat, active decay, advanced decay and skeletonization. The fresh stage begins once the person has died and continues until the second stage starts. Bloating refers to the inflation of the body caused by gases that are produced by microbial activity. The rate of decomposition increases in the active decay stage and is contributed to by insect activity. Once the insect activity has reduced and a majority of the flesh has been removed, the body is considered to be in the advanced decay stage. During the skeletonization stage, the body is primarily a skeleton, besides dry skin and hair being present (Adserias-Garriga et al. 2017).

Time is not the only factor that effects PMI estimation methods. Environment also effects PMI such as insect activity, climate, microbial activity and scavengers (Adserias-Garriga et al. 2017). Environmental factors can also be beneficial to PMI estimation. Traditional methods, like those detailed above, only work for short term PMIs. The environment around a corpse may be used for longer PMIs. The use of soil analysis, insect activity, and plant material for estimating PMI will be outlined in this paper.

**Soil Analysis**

**Soil Biology**

 Microorganisms are found everywhere and in a majority of habitats. Microorganisms use humans as a host and are comprised of multispecies communities. When a human dies, these communities change fast. Soil under a cadaver also changes as the body decomposes due to bodily fluids and microbes seeping into the grave soil. These changes in soil microbes allows for its use in PMI estimation. Finley et al. (2014) describes the decomposition stages as a continuum and not a discrete series. This allows for microbial communities to be used for a more precise PMI. There is a shift from aerobic to anaerobic microbes during the bloat stage. Anaerobic microbes outweigh aerobic microbes until the carrion ruptures. After rupture, microorganism flow out of the body and drain into the soil beneath the cadaver (Finley et al. 2014). The area in which the microbes and other bodily material is found around a cadaver is called the cadaver decomposition island. It also includes the changes in soil biology and chemistry. Remnants from the decomposed body dwell in the soil for months post-death (Adserias-Garriga et al. 2017). However, microbial diversity of the cadaver become similar to the soil microbial diversity as the cadaver reaches the last stage of decomposition.

 The following case studies will outline three different ways microbes or Volatile Fatty Acids may be used for PMI estimation. Case one looks at the use and changes of *Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes* in soil samples. The next study compared untreated and treated soils and found the presence of *Acidobacteria, Proteobacteria*, *Rhodospirillales, Kaistobacter* and *Xanthomonadales*. Lastly, the final case study examined Volatile Fatty Acids and their uses for PMI estimation.

**Soil Microbe Study at the University of Tennessee:**

 Adserias-Garriga et al. (2017) collected soil samples at the University of Tennessee Anthropology Research Facility from three cadavers. Samples were taken during the different stages of decomposition up until the advanced decay stage. The cranial, abdomen and feet areas of the cadavers were used for sampling. Each sample was taken daily and at the same time of day for consistency. Control soils were taken at the same time as the other samples from a distance of one meter from the cadaver.

 Changes in cadaver bacterial communities were similar in all three bodies. Samples from days 1 through 6 which ranged from fresh to bloat stage (donor 1) to fresh to active decay stage (donor 2 and 3) contained bacterial community naturally occurring in the soil. This includes *Proteobacteria, Acidobacteria, Bacteroidetes* and *Actinobacteria* (in lower quantities than the previous three). *Firmicutes* increased on days 6 and 7 which was associated with a decline in *Proteobacteria*. *Actinobacteria* also increased. All bacteria found naturally in the soil prior to decomposition also declined over these two days.

Presence of *Firmicutes* continues after the 6 to 8-day range. *Firmicutes* are found in samples from the cranial and abdominal area in earlier stages of decomposition than in samples from the feet area. Two orders of *Firmicutes* are important to note. The order *Clostridia* appears earlier than *Bacilli*. Within the order of *Clostridia*, *Clostridiaceae* arrive first but decrease during the skeletonization stage. *Clostridiales* appears later than *Clostridiaceae*. *Bacilli* varied between donors, with *Bacillaceae* in all of the donors. *Veillonellaceae* and *Planococcaceae* was only found in donor 1. This drastic change from indigenous soil microbes to an increase in *Firmicutes* during days 6 to 8 are “probably as a consequence of the transfer of body decomposition microbiota to the soil” (Adserias-Garriga et al. p. 393.) *Firmicutes* is important to forensic investigations because according to Adserias-Garriga et al. research, the bacteria is correlated with the bloat and active decay stages.

**Soil Microbe Study at Texas State University:**

Soil samples that were both untreated (without buried remains) and treated (with buried remains) were obtained from the Forensic Anthropology Research Facility at Texas State University. The total samples consisted of two untreated and four untreated soils. The cadavers were in the last decomposition stage, dry remains, when the study was conducted. Eighteen total soil samples were taken.

 Across both treated and untreated soils, *Acidobacteria* was most abundant. The study also showed that *Proteobacteria*, *Rhodospirillales* and *Kaistobacter* were the lowest amounts of bacteria present in both treatments. *Xanthomonadales* were found in the treated soils, however, it was not found in the untreated soil samples. The results, which were taken at longer time-scales, shows that the diversity of microbes in the soil become homologous in the treated and untreated soils. This confirms the idea that as time passes, the exchange of microorganisms between the corpse and the soil comes to a halt (Thomas et al. 2017).

**Volatile Fatty Acids:**

 A study on Volatile Fatty Acids (VFAs) was conducted at the University of Tennessee Anthropology Research Facility on seven cadavers. Each was placed at the facility at varying times during the year. Soil samples were taken every three days during the spring and summer and switched to weekly sampling once the dry remain stage was reached. Samples were taken weekly during the fall and winter because bodies decay at a slower rate in cooler weather. VFA’s consist mostly of broken-down muscle and fat. There are many organic compounds included under VFAs, however, only seven are detectable in soil solutions. The seven detectable are formic, acetic, propionic, butyric, valeric, caproic, and heptanoic acids.

 Propionic, butyric and valeric acids form and go into soil solutions at a specific ratio (Vass et al. 1992 p. 1245). The acids come from the decomposing cadaver and it release is temperature dependent. Due to the specific ratios and temperature dependent release, propionic, butyric and valeric acids may be used for PMI. VFAs and decomposition stages show a correlation according to Vass et al. (1992). The fresh stage does not produce much VFAs change. When the carrion ruptures during the bloat stage, fermented by-products seep out and are rich in butyric acids. VFAs were seen to be at their highest rates after maggot migration because insects cut off the flow of bodily fluids leaking into the ground. The study also found that the release of VFAs stops at 1285 ± 110 Accumulated Degree Days (ADD). To find PMI based on ADD, an investigator may divide 1285 (when the dry remain stage occurs) by the average °C on the day the remains were found. Unlike a majority of PMI methods, this equation gives a maximum PMI instead of a minimum (Vass et al. 1992).

 There are a few variables that investigators need to consider based on this study. Insects consume a cadaver at a slower rate when the temperature drops. If there are less insects cutting off the flow of VFAs production then more VFAs are produced and in turn, more end up in the soil (Vass et al. 1992). This would increase the percentage of VFAs in a soil sample and make it harder for investigators to match the PMI times to this study. Additionally, a decrease in temperature, below 4°C, makes it more challenging to pinpoint PMI. If soil samples exhibit high rates of caproic and heptanoic (long chained VFAs) then an investigator will be able to determine that a corpse was in colder temperatures.

**Summary:**

 The experiments conducted by Adserias-Garriga et al. (2017) (case 1) and Thomas et al. (2017) (case 2) found similar bacteria in their studies. *Proteobacteria* and *Acidobacteria* were found in both studies but in different quantities. *Proteobacteria* was found in lower amounts in the Thomas et al. (2017) study while *Proteobacteria* was one of the three main bacteria found in the untreated soil in the Adserias-Garriga (2017) study. *Acidobacteria* was most abundant in case 2 while it was almost equally abundant as *Proteobacteria* and *Bacteroidetes* in case 1. Between both studies, numerous bacteria were mentioned in on and not the other.

*Actinobacteria, Firmicutes, Clostridia* and *Bacillaceae* were studies in case 1 but not case 2.

*Rhodospirillales, Kaistobacter* and *Xanthomonadales* were present in case 2 but not discussed in case 1. Both of these cases revealed important information on using soil microbes for PMI estimation. Case 1 offers valuable information for short PMIs while case 2 is relevant for longer PMIs.

 Case 3 (Vass et al. 1992) is not comparable to case 1 and case 2 due to it using VFAs and not soil microbes. VFAs are very useful to PMI estimation due three VFAs (propionic, butyric and valeric) absorbing into the ground in specific ratios. VFAs also show correlation with decomposition stages that are outlined in the introduction. See reference table at the end of the paper for more detail. A pivotal point in the study came when Vass et al. (1992) found that VFAs cease to be released at 1285 ± 110 Accumulated Degree Days. Overall, all three cases supplied important information for forensic investigators when estimating PMI.

**Soil Chemistry:**

 Fancher et al. (2017) conducted a study on the use of soil chemistry for estimating PMI. When a cadaver is in the process of decomposition the soil chemistry around the body changes. The cadaver decomposition island (CDI) begins to be infiltrated by volatile and persistent compounds (Fancher et al. 2017 pp. 130) throughout the different decomposition stages. Nitrate-N, ammonium-N and dissolved inorganic carbon (DIC) were found to be at their highest rates early on in the decomposition process and declined to control values as time proceeded. For later PMIs, a forensic investigator may use dissolved organic carbon (DOC), dissolved organic nitrogen (DON), orthophosphate-P, sodium and potassium. The study found that these are in abundance above the control for up to 1752 days after death.

 Two control soils from the Forensic Anthropology Research Facility in San Marcos, Texas were used for the experiment and are denoted at RUD and CrD. Electrical conductivity may prove useful for PMI estimates. In both RUD and CrD soils, electrical conductivity increased as time passed up to 694 and 684 days, respectively. There was a spike in sodium in the RUD soil at 35 days and increased to ambient levels at 767 days (Fancher et al. 2017 pp. 133). Potassium levels rose early in both soils; however, it rose at different rates. CrD has increased potassium at 14 days versus 33 days in RUD. Nitrate-N concentration intensified later than sodium and potassium at 272 days in CrD and 171 days in RUD. It returned to normal concentrations at 648 days in RUD and 854 in CrD.

 Dissolved organic carbon rates increased tremendously within the first month. The rates in CrD and RUD soils varies greatly with DOC increasing six times in concentration within only 6 days. Concentration increased took 5.5 times (33 days) as long in RUD but multiplied by 13 times. As with DOC, dissolved organic nitrogen days until peak varied considerably. At 263 days, DON concentration was at its greatest in CrD while it took only 132 days in RUD soil.

**Soil Chemistry and Entomology:**

 According to Aitkenhead-Peterson et al. (2015), forensic entomology is the best PMI estimation method. Cadaver and soil chemistry play a role in insect activity. Chemicals from the cadaver are used by insects for multiple reasons including energy and nutrients. Cadaver chemicals also attract insect to the body and effect insect succession. Many of the chemicals that attract the insects are volatile organic compounds but there are various others. Insect succession for PMI can be used because insects and microbial communities change as the chemicals in the cadaver change.

 The chemicals in the cadaver are released into the surrounding ground becoming a part of the CDI. A study by Hoffman et al. (2009) looked at concentrations of volatile organic carbon (VOC). In the early stages of decomposition, the VOCs most found were alcohols, aldehydes, cyclic and halogenated compounds. As decomposition continued aldehydes continued to be present but alkanes were also detected. Studies by various authors (Carter et al. 2008; Van Belle et al. 2009; Spicka et al. 2011) found that ninhydrin reactive nitrogen (NRN) could be used for PMI estimation based on rat and pig cadavers. Van Belle et al. (2009) found that NRN was in more concentrated in the treated soil after 3 days than in the untreated soil. This increase remained until the end of their 97-day experiment. NRN reached its maximum at 14 days and leveled out at 60 days (Aitkenhead-Peterson et al. 2015 pp.286). The concentration of NRN did decline but not until the end of the experiment.

**Forensic Entomology**

 The use of insects for PMI estimations is a common method backed by well-established science (Aitkenhead-Peterson et al. 2015). Forensic entomologist uses the age of an insect to estimate PMI from the first day after death to several weeks after death. Insects are drawn to a body usually immediately after death (Amendt et al. 2004). Usually they are attracted by the chemicals released by decomposition. The age of an immature insect may be used because adult flies rarely lay eggs on a living body (Wells and Lamotte 2001). There are two methods that Forensic entomologist use when predicting PMI from insects. First, they may look at the succession of a particular insect and its pattern of colonization. Second, the life cycle and rate of development of flies can be analyzed. As with many other PMI estimation methods, this method depends on climate, region, burial method, animal activity, etc (Aitkenhead-Peterson et al. 2015). Forensic entomologist may also look at insect remains or evidence left by prior insects on a body at the time of examination. Additionally, the lack of a specific species that is expected to be present, such as blowfly larvae, is important for the forensic entomologist to note (Anderson 2001).

 According to Wells and Lamotte (2001 pp. 265), “the most important implication for PMI estimation is that carrion insect species differ in terms of growth rate, arrival time, and position within the order of succession.” Another essential factor in using insects for PMI estimation is preservation. PMI is estimated counting backwards from the point of preservation of the specimen, thus the time of preservation should be documented appropriately (Wells and Lamotte 2001). There are various issues when using insects for PMI estimations. Blowfly growth rates differ depending on the type of tissue they consume and circumstances prior to death, such as an overdose, may affect these rates (Aitkenhead-Peterson et al. 2015). Temperature and burial type play a huge role in insect timing and succession. Some species avoid certain ranges of temperatures and buried bodies in enclosed spaces make it harder for insects to reach the cadaver. These issues skew PMI estimations and can lead to estimations higher or lower than those that would be normally estimated (Aitkenhead-Peterson et al. 2015).

 Blowflies and various species of beetles are typically used for PMI estimations. Forensic entomologist should avoid using any insects that have reoccurring life stages. Insects that leave and return to the cadaver various times are also not useful for PMI estimation (Wells and Lamotte 2001). Insects are attracted to cadavers due to chemical responses (see Soil Chemistry and Entomology section, paragraph one, for details). In addition to chemical attraction, insects may be attracted to a cadaver for a food source or a location to lay eggs (Anderson 2001). The species that arrive early are “from the orders Diptera (flies) and Coleoptera (beetles)” (Amendt et al. 2004 pp. 53).
**Calliphoridae (Blowflies):**

Blowflies are among the first insects to be drawn to a body, enticed by the odor of decomposition. These insects can be used to estimate PMI for two weeks or more (Amendt et al. 2004). Once a cadaver has reached the point of mummification, blowfly attraction to the body ceases. Arrival time of blowflies depends on genera and species of the particular fly. Lane (1975) found that in England, *Calliphora vicina* and *Lucilia caesar* were attracted to rodent remains within minutes or hours after death. *Lucilia illustris* did not appear on the cadaver until 76 hours after death in a woodland setting and 48 hours in open grassland. In a study done in Missouri, *Phormia regina* were low in abundance 24 hours after death, however, increased in number as time went on, reaching a greater abundance at 72 hours after death. As with *P. regina*, the study found that *Cochliomyia macellaria* were found in later stages after death. They arrived 18 to 48 hours after the cadaver had died (Hall and Doisy 1993).

The condition of a cadaver effects whether a female blowfly will oviposit (lay eggs) or not. Eggs require moisture to survive and develop properly so a female will not lay on a dehydrated cadaver or in its dry remain stage. Open wounds and orifices are where oviposition starts. As stated previously, burial remains affect blowfly colonization. Blowflies are not able to bury deeper than 30 cm (Introna and Campobasso 2000; Campobasso et al. 2001**)** which makes deposition eggs on buried remains difficult. This effects arrival time of blowflies and alters PMI estimations (Amendt et al. 2004). Time of day also influences oviposition as blowflies are diurnal. Despite not laying at night, blowflies will lay eggs when the cadaver is in a dark area. Oviposition is also temperature dependent as blowflies do not lay below 10ºC (Anderson 2001).

 Insect succession is a useful tool for PMI estimation. “A succession model includes information about the time elapsed between death and the appearance of a particular arthropod species and stages” (Wells and Lamotte 2001 pp. 264). This allows for it to be used for not only minimum but also maximum PMI (Schoenly et al. 1992). As with other methods, the use of succession varies by region and season. Insect age is important to PMI estimations. The use of the immature stages of insect life cycles may help estimate PMI starting at one day and continuing past one month (Amendt et al. 2004). Positive identification of the exact species of an insect is a crucial first step forensic entomologists must do. In order to find minimum PMI, the immature larval stage of an insect life cycle must be found. Length and dried weight of the oldest larva preserved can give an age by referring to reference data. Developmental stages of each insect have its own requirements and the number of degree-days needed to reach full development varies by species (Amendt et al. 2004).

**Coleoptera (beetles):**

 As previously mentioned, blowfly activity will cease once a body reaches its dry remains stage in the decomposition process. The main insect used during and after the dry remain stage are from the order Coleoptera (beetles). Beetles are the largest group of insects and the order Coleoptera has numerous groups that are useful to forensic entomology. The families in Coleoptera used for forensic purposes are Staphylinidae, Scarabaeidae, Carabidae, Histeridae, Silphidae, and Dermestidae. Dermestidae (skin beetles) are one of the most valuable families. They are small and oval shape, with the largest species measuring 0.8mm. Skin beetles are characterized by their pale grey or brown markings. Females oviposit 150 eggs which hatch within three weeks. “Larval stages last from 5 to 15 weeks” (Kulshrestha and Satpathy 2001 pp. 16). As with other insects, skin beetle’s development depends on temperature and food sources available. Anderson and Vanlaerhoven (1996)found that skin beetles arrive 21 days after death, around the time some amount of flesh was still present. Abundance of skin beetles increased at 43 days. Adult skin beetles were collected early on (3-5 days) however, no larvae were present at this time. Adult insect activity is irrelevant to PMI as only larvae represents to total species infestation.

**Geographical Variation of Insects:**

 Individual species of insects can vary by region. *Chrysomya* spp. is generally found in southern regions of United States and other subtropical regions. This species is absent in Western Canada and rare in other parts of Canada. Ontario is an exception to this, as one species is found there. Not only do species vary by regions but timing of arrival also varies. General groups, such as Calliphoridae (blowflies), will still appear first but the species will change (Anderson 2001). Studies by Early and Goff (1986) and Reed (1958) illustrate this variation. In Hawaii, some of the first colonizers are *Phaenicia cuprina,* *Chrysomya megacephala*, and *Chrysomya rufifacies* (Early and Goff 1986). In contrast, *Phaenicia coeruleiviridis* and *Phormia regina* were found to arrive first in Tennessee (Reed 1958). It should be stressed that due to the variation in species and arrival time by region, data from one region should not be applied for PMI estimation of a different region (Anderson 2001).

**Season variation of Insects:**

 Season may have an effect on some species life cycles. For example, larvae may enter a stage of dormancy in response to seasonal changes. This lengthens the time spent at the larval life stage (Wells and Lamotte 2001). Seasons alter blowfly abundance in many species. Goddard and Lago (1985) found that *Phaenicia coeruleiviridis* and *Cochliomyia macellaria* were in greater in abundance during the summer. On the other hand, *Calliphora livida* and *Cynomyopsis cadaverina* were the abundant species in the winter time. Carrion insects may also reach their peak activity at various seasons throughout the year. Time since death may have no relation to some species time of arrival. Seasonal changes may be the cause for time of colonization in some species of carrion insects. Anderson (2001) reached two important conclusions from the effects of seasons on insects. Time of arrival should be studies during all seasons to capture accurate data of colonization time. Additionally, forensic entomologist may be able to determine not only PMI but also season of death from insects.

**Rural vs. Urban Setting:**

 Another consideration to take into account when using insects for PMI estimation is rural versus urban variation. The differences in urban and rural species can be seen in their food source. Rural species eat the flesh of dead animals and urban species live on discarded food.

Blowflies species are specific to rural and urban settings. Not only can this type of environmental variation be used for PMI estimations, but it can also tell a forensic entomologist if a body has been moved. Not all blowfly species are fixed to rural or urban environments, many are found in both. An example of this variation comes from a study in British Columbia. Anderson (1995)concluded that *Protophormia terraenovae* and *Calliphora vomitoria* were rural species while *Polyphaenis sericata* were only in urban areas.

**Forensic Botany**

Botanical evidence can supply extra evidence for PMI estimations, but the ability to use this evidence starts at the crime scene. Investigators must be knowledgeable in recognizing important plant material, documenting its location, establishing its relationship to the surroundings and collecting and preserving the material properly. This is also the case with insect material and collection. Although collection of evidence is easy, recognizing important evidence can be a challenge. Untrained investigators may hinder the collection of botanical evidence.

 Locating and connecting large pieces of botanical evidence (a branch) to its surrounding is a something an untrained investigator may be able to do. However, microscopic botanical evidence (pollen) may go unnoticed by the untrained eye. Another issues an uneducated investigator may come across is whether or not the botanical evidence is ingenious to the area or its rarity. Coyle et al. (2005) suggest that “when in doubt, plant material should be collected along with all necessary control samples” (pp. 607). Proper packaging of botanical evidence, as with all evidence, is crucial. Packaging can vary depending on the material of the evidence but in general, botanical evidence should be packaged in paper after drying. This prevents the growth of bacteria and excess moisture that can damage the specimen. Evidence should be packaged separately to avoid cross-contamination (Coyle et al. 2005).

**Determining Manner of Death Case Study:**

 Coyle et al. (2005) present a case study that illustrates how botanical evidence may be used for determining manner of death. In Taipei, Taiwan, a woman’s body was found lying in a gutter. Prior to the autopsy, it was thought that the woman died from a hit and run accident. Video surveillance showed her present prior to a truck moving in front of her and then disappearing after the truck was gone. A tiny berry and stem were found in the victim’s hair during the autopsy. This flora was not indigenous to the area.

Back at the crime scene, another part of a broken stem was found where the body had been lying. After observing potted plants hanging on a railing above, they proceeded to identify the potted plant. It was a match for the plant material found in the victim’s hair. Due to the weather the day of the incident, investigators concluded wind could not have broke the stems. Instead, they decided there must have been some type of impact to break the plant stems. Due to the height of the railing (3.5m), an individual passing by could not have reached the potted plant. Investigators realized that it was likely the impact of the woman falling that broke the plant. After the autopsy and speaking with the family, the death was deemed a suicide (Coyle et al. 2005).

**Determining if a Body was Moved Case Study:**

 Botanical evidence can “be used to link a body or a weapon back to a primary crime scene” (Coyle et al. 2005 pp.609). The body of a man was found in a gutter in Taiwan holding botanical material in his hand. Upon observation, it was noted that the victim had many injuries and contusions below the knees. An autopsy was performed and a piece of bamboo was extracted from his stomach. No bamboo was found at the location of the crime scene. The bamboo was then used to find the original crime scene where the injured victim was trying to stay alive. The gutter was the secondary scene where the man had died attempting to find help.

**Plant Succession as Botanical Evidence Case Study:**

 Disrupted patches of earth, usually in regards to burials, will follow a set pattern of plant succession. The first colonizers of disturbed land are grass, small shrubs and trees then follow. PMI of these graves can be determined based on plant succession. Investigators must take into account the amount of sunlight and soil conditions when making these estimations. As with a majority of PMI estimations methods, plant succession can only give a minimum PMI and not a maximum. An investigator cannot reach a maximum PMI because it is almost impossible to know when the plant seed was set and began to grow (Coyle et al. 2005).

**Botanical evidence from a wooded environment:**

 Remains left on the ground in a wooded environment will integrate into the surroundings as time passes. Green algae, mosses, lichens and fungi are some of the botanical material that can grow on bone. Roots and shrubs may grow through the bones and clothing over time. Cardoso et al. (2010) states that botanical material is useful for PMI estimates because the plants are permanently attached to the substrate and pattern and growth rate identification is easy (pp.452).

As stated previously, this method can only yield a minimum PMI. A minimum PMI does not provide a lot of information however, it can help include or exclude possible victims.

 The remains in this study were in the skeletonization stage. Botanical evidence was collected from both the skeleton and the clothing. Several roots had grown through the disarticulated skeleton. The ribcage still contained clothing and was bound by roots. Roots also bound other parts of the remains. Green algae and moss were found on some of the bones and clothing but not on all parts of the body. Due to the remains being in the skeletonization stage, no insect activity was found. The study collected and examined two types of botanical evidence, bryophyte (mosses) and vascular plants (shrubs). Growth of bryophytes can be determined by looking at their annual segments along the stem. Age for vascular plants are estimated by growth rings in the roots. The investigators were able to concluded that the minimum PMI for the remains was three years.

**Plant succession continued:**

 Human remains were found in Perugia, Italy in November 2010. The environment they were found in was a wooded area with many streams. Partial remains were collected and were skeletonized. Observation of the remains at the lab showed a bryophyte colony inside the lower cranium. The annual segments on the stems were counted to obtain and age. This age was between twenty-four to thirty months. The age of the moss is then equal to the age of minimum PMI.

**Conclusion**

Various different methods for determining PMI have been illustrated throughout this paper and can be summarized in the following way. For PMI estimates immediately after death, postmortem lividity can be used. Algor, livor and rigor mortis are the optimal methods for use after an hour after death has been achieved. Livor mortis may be used an hour after death while rigor mortis can be utilized two to six-hour postmortem. Once twenty-four hours have passed, the traditional methods for PMI estimation may no longer be applied. Soil microbes surrounding the cadaver may be used for up yo twenty-four to forty-eight hours after death. Volatile Fatty Acids may be useful for PMI’s of four-ten days. When the PMI extends beyond seven days, three other methods may be employed. Soil chemistry and blowfly larvae may be studied to estimated PMI’s starting at fourteen days after death. For long PMI’s beetles are a useful insect to examine to achieve an estimate. Unlike the previously mentioned estimation methods, botanical evidence does not have an estimated starting time for use. The use of botanical evidence is individual to each case and the starting time of use depends on the genus and species of the plant in question. The details listed here may also be seen in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Method:** | **Start time:** | **Typical end time:** | **Signs:** | **How to use:** |
| Postmortem lividity  | 20-30 minutes AD\* | 8-12 hours AD | Pink to dark pink/blue patches | Apply pressure to remove lividity1 |
| Algor Mortis | ---- | ---- | ---- | Take the temperature of the cadaver and subtract 1.5°F from 98.6ºF which gives hours since death |
| Livor mortis  | 1-hour AD  | Up to 9-12 hours AD | Pooling of blood in areas closest to the ground | Visually exam pooling of the blood |
| Rigor Mortis2 | 2-6 hours AD | Up to 24-48 hours AD | Stiffening of the body  | Examine degree of stiffness |
| Soil microbes | 1-2 days AD | ~50+ days AD 3 | ---- | Testing soil samples from under the cadaver |
| Volatile Fatty Acids | 4-10 days AD | 1285 ± 100 ADD9 | ---- | Testing soil samples from under the cadaver  |
| Soil chemistry4 | ~14 days AD5 | ~1752 days AD6 |  |  |
| Blowflies  | 14 days AD | ~25-50 days AD | Insect larvae or adult insects on cadaver | Collect larvae of different species off the cadaver  |
| Beetles | ~50+ days AD | ~50+ days AD | Insect larvae or adult insects on cadaver | Collect larvae of beetles off of the cadaver |
| Botanical material | ----7 | ----8 | Roots, moss, or other botanical material in or on the cadaver  | Collect samples of the botanical material |

\* after death

1 as time progresses, pressure will not relieve lividity

2 along with livor and algor mortis,rigor mortis does not apply if soft tissue decay has begun

3 length of use varied by case study. See Soil Biology for details.

4 based on Fancher et al. (2017) case study

5 dependent on which chemical is tested

6 dependent on which chemical tested

7 dependent on which botanical material is being examined

8 dependent on which botanical material is being examined

9 accumulated degree days