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ORIGINAL ARTICLE

Post mortem changes in relation to different types of clothing

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Abstract

Post mortem changes are important in estimating post mortem interval (PMI). This project's aim was to study the effect of burial and type of clothing on rate of decomposition, which can contribute to estimating PMI for victims. 12 rabbits (*Oryctolagus cuniculus*) carcasses were separated into 3 groups: no clothing, light clothing and heavy clothing. Control subjects were placed on the ground surface while test subjects were buried at 30 cm depth graves. Soil samples prior and after decomposition were collected for soil pH and moisture analysis. Post mortem change was assessed using a Total Body Score system. The head, neck and limb regions were found to decay faster than the body trunk region. Mummification occurred on body parts that were exposed directly to the atmosphere while adipocere formed on some buried subjects. Burial delayed decomposition due to lower insect activity and lower soil temperature. The soil layer also blocked the accessibility of majority of the arthropods, causing further delay in decomposition. Clothing enhanced decay for bodies on ground surface because it provided protection for maggots and retained moisture on tissues. However, clothing delayed decomposition in buried bodies because it physically separated the bodies from soil and arthropods. Higher sun exposure and repetitive exhumation showed acceleration of decomposition. The decomposition process increased soil pH and moisture percentage values. Soil pH initially increased until pH 8.0-8.4 followed by a slight decrease while soil moisture percentage changed inconsistently. Burial was significant in affecting post mortem change, $F(1,11)=12.991$, $p<0.05$ while type of clothing was not significant, $F(2,9)=0.022$, $p=0.978$ and combination of both type of clothing and burial factors were also not significant, $F(2,3)=0.429$, $p=0.686$. For validation, an accuracy of 83.33% was achieved based on soil pH and soil moisture percentage analysis.

Keywords: post mortem change, decomposition, burial, clothing, soil, TBS system

INTRODUCTION

Post mortem interval (PMI) is the amount of time that had passed after a person dies and post mortem changes are important for forensic pathologists in estimating PMI for the deceased. Unfortunately, the development of post mortem changes is subject to various environmental factors. It is a process where bio-molecular structures of an organism becomes less stable after chemical or biochemical degradation and provide energy for other new organisms.[1] Cellular death results in physical visual changes until dry remains. Degradation is mainly based on 2 mechanisms i.e. autolysis and putrefaction. Autolysis is cellular self-destruction by enzymes present intracellularly while putrefaction is decay of organic substance by bacteria and other microorganisms. Bacteria from the gastrointestinal tract will spread all over the body through blood vessels and the lymphatic system and degrade the complex organic material into simpler gases, fluid and salts.[2]

Decomposition can be first noticed by green discolouration of the abdomen wall, and swelling of the face and abdomen due to build-up of decompositional gases and fluid.[3] Oviposition by arthropods could be found on natural orifices or wounds that are present. The body then deflates with purging of gases and fluid with massive maggot activity. Fauna succession then progresses to wider variety of species including spider and beetle.[4] However, bodies stored under special

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conditions may show variations. Mummification is dehydration of tissue that retards putrefaction due to low moisture content.[5] Adipocere formation requires moist, anaerobic conditions where partial degradation of fat forms greyish to yellowish grease substance on body surfaces.[6] Both mummification and adipocere formation can occur in localized body parts instead of the whole corpse and provide preservation of tissues.[2]

Bodies buried in graves decay slower than bodies on ground surface because the soil layer delays arthropod and scavenger animal accessibility. Poor gas diffusion in graves reduce the variability of macro and microorganisms that are present.[7] The deeper the grave, the slower the decomposition. Bodies buried in 30 to 60 cm depth take months or up to a year to reach the dry bones stage while those at 90 to 120 cm need years to reach the same stage.[8] However, arthropod activity can still be observed on bodies in shallow graves at 30 cm depth because arthropods can squeeze through the small cracks of the soil layer to gain access to the body. Some may oviposit on ground surface and eggs and larvae move downwards by rainfall diffusion.[9] The first specific objective of this study is to study the effect of burial on carcasses.

Clothing has different effects on decomposition based on its material and thickness. Light clothing enhances decomposition by protecting maggots from sunlight, rainfall and other hazards.[10] But with increasing amount and layers of clothing, more time is required for maggots to reach the body.[11] Clothing made of cotton or other natural materials are susceptible to microorganism degradation while artificial polyester clothing is resistant to degradation.[12] In burial conditions, clothing enhances formation of adipocere because it retains the moisture released from decaying tissue thus creating a consistent wet environment.[13] The second specific objective of this study is to observe the effect of clothing types on carcasses while the third specific objective combines both burial and type of clothing parameters and their effects on the bodies.

This study is important to understand the influence of burial and types of clothing in affecting post mortem changes on a dead body. Burial method is commonly used by murderers to hide their crimes and the depth of graves are about 50 cm.[14] A local study showed that decomposition in Malaysia occurs faster than other countries due to climate, forensic entomology and geographical differences. According to Chin,[15] a swine carcass needs 8 days to reach the dry remains stage in a local oil palm plantation while the same process needs 25 days in Hawaii and 40 days in Western Australia. Besides that, there is a dearth of study regarding the combination of burial and clothing factors in affecting the rate of decomposition.

MATERIALS AND METHODS

In this research, 12 adult rabbit (*Oryctolagus cuniculus*) carcasses weighing 2-3kg were separated into 3 groups: without clothing, light clothing and heavy clothing. Light clothing include T-shirt and short pants while heavy clothing include long sleeve shirt, long pants, jacket and stokings. One subject in each group was chosen as control, labeled KX (Control-without clothing), KN (Control-light clothing) and KT (Control-heavy clothing) and placed on ground surface. The remaining rabbits were test subjects labeled from 1 to 9 and buried at separate 30 cm graves with own respective clothing setting. Review and approval were granted by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

Intervals of observations

Before placement or burial of subjects, soil samples were collected. The distance between all graves were 5m apart to avoid any interference from one another while ensuring identical microclimate.[11] The control group was observed daily until they reached dry remains stage while test subjects were exhumed at certain time interval. Subject 1 was exhumed at second week after burial for observation then reburied at the same grave. On fourth week post-burial, Subject 1 and 2 were exhumed together and then buried back while on sixth week post-burial, all three Subject 1, 2 and 3 were exhumed and observation were completed. The same criteria were applied to other two clothing groups.

Post mortem changes

Post mortem change was assessed with using a Total Body Score (TBS) system, dividing the carcass into 3 sections, i.e. (i) head & neck, (ii) body trunk and (iii) forelimb & hindlimb, as it is known that different parts of the carcass decay at different rates.[16] Using the TBS system, each section has independent scores based on its respective visual post mortem changes and the sum of marks from 3 sections is the TBS score for the subject, reflecting the current stage of decomposition.

The TBS score were analyzed with SPSS using Two-way ANOVA analysis for the first two specific objectives to study the differences between control and test subjects and also between each group of clothing setting. Multiple-way ANOVA was then used for the third specific objective to determine the combination effect of burial and clothing. Throughout the study, ambient temperature, relative air humidity, rainfall and soil temperature were collected on specific days.

For control subjects, soil samples were collected at 5 days intervals while soil samples for test subjects were collected in first exhumation only. Soil properties analysis included soil pH and moisture content. For soil pH analysis, 10g of soil sample was added with distilled water at ratio of 1:2.5[7] and water portion was separated after it had homogenized for pH value determination. For soil moisture analysis, 10g of soil sample was heated in a furnace at 105ºC for 24-hours and then reweighed for moisture percentage calculation.[17]

RESULTS

Controls

The development of TBS scores for control subjects is shown in Figure 1. Control subject KX was initially colonized by ants on day 3 and 4. On day 5, the head and neck region showed caving in appearance with bone exposure on day 6. The body trunk was bloated on day 4, fur detachment occurred on day 6 and caving in appearance occurred on day 8. However, the skin of the body trunk underwent mummification on day 9 with dark brown colour slowly fading into light brown by the end of the study. The mummification caused the region (ii) score to stop at 8 since day 9. For the forelimb and hindlimb region, bone exposure started on day 5 and small amounts of hardened skin still could be observed at the joints by the end of study. The total TBS score for KX was 30 at day 10. Before body disposal, the mummified skin was cut through and there was full skeletonization of the body trunk under the skin with no internal organ preservation observed.

Subject KN was the only subject that reached the full TBS of score 35. It also showed ant colonization at the start of the study. The head and neck region was bloated on day 4 and bone exposure started on day 6. Bloating of body trunk started on day 4 and fur detachment on day 6. Bone exposure was observed on the next day starting from the rib cage. For the forelimb and hindlimb region, fur detachment started on day 4 and bone exposure on day 5. However, scavenger animal disturbance was observed on days 8 and 9 when clothes and part of the body were lost.

The head and neck region of KT recorded fur detachment on day 3, bloating on day 4 and caving in appearance on day 6. Bone exposure started on day 8 and full exposure on day 10. The body trunk was bloated on day 4, fur detachment

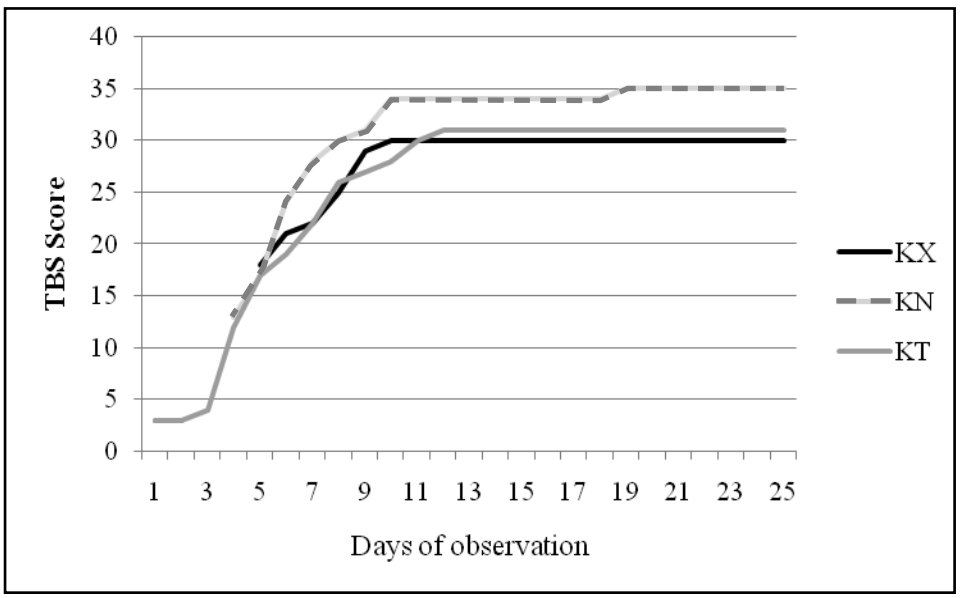

FIG. 1: Development of TBS score for control subjects.

on day 5 and caving in on day 6. The tissue formed a layer of melted appearance of greyish green colour slowly turning into brown colour. Bone exposure started from day 7 while the tissue slowly dried up and hardened on day 18. The tissue was flipped over by the end of study and it showed full bone exposure with waxy appearance and no internal organ preservation. For limbs, fur detachment started earlier at the forelimbs on day 4 while at the hindlimbs on day 5. However hindlimbs entered dry remains stage on day 7 while the forelimbs were still in advanced decomposition until day 11. The total TBS score reached was 31.

Test subjects

Among the test subjects, head, neck and limb regions decomposed faster than body trunk regions. Decomposition of body trunk showed a trend of post mortem changes. Starting from bluish-black skin, inner decaying tissue which was initially pink or white in colour, turning into dark red or brown; finally into black before a high degree of bone exposure was observed. The pelvis region decomposed slower compared to thoracic and abdominal region. The TBS scores are shown in Table 1. White creamy substance with adipocere characteristics was found in subject 4, 5 and 7 but in very small quantity.

Insect activity and mould

Generally, entomological insect activity on subject KX and KN were low. Larvae activity on KX and KN both stopped on day 9 and day 8 respectively while KT recorded the longest larvae activity - up to day 17. For test subjects, larvae activity was only observed at exhumation 2 weeks post burial. At the end of this study, all control subjects showed high degree of bone exposure; and colony of white mould was found on all 3 control subjects. The comparison of ambient temperature and soil temperature is as shown in Figure 2.

Clothing

From the clothing aspect, clothes of KN were wet before these went missing and the same condition was found on the long sleeve shirt and pants of KT although they gradually dried up and hardened. The head portion of the jacket caused accumulation of detached fur and decomposed tissue thus the skull of KT was "buried" among it. However, the presence of the jacket allowed some post-feeding larvae to transform into pupa. Clothing on all test subjects were wet and the delayed effect on decomposition could only be observed after 2 weeks post burial. The difference became less pronounced until it almost disappeared on 6 weeks after burial.

Statistical analysis

SPSS analysis showed that the burial factor was significant in affecting the TBS score, $F(1,11)=12.991$, $p<0.05$ with observed power of Day*Burial factor was 0.906 or 90.6%. However type of clothing did not show significant differences among types of clothing, $F(2,9)=0.022$, $p=0.978$ and combination of burial and type of clothing factors also was not significant, $F(2,3)=0.429$, $p=0.686$.

Soil analysis

For soil pH analysis, all control subjects started with acidic soil with pH 4.7–6.0 which generally increased as the study progressed. Soil pH increased up to 8.0–8.5 followed by a slight decrease recorded at pH 7.9–8.1 as shown in Figure 3. Soil samples in shallow graves started with acidic soil, pH range 4.8–5.8 and all increased to pH 5.6–8.4 as shown in

TABLE 1: TBS score for buried test subjects

Test subjects	Total TBS score after burial		
	Second week	Fourth week	Sixth week
1	22	31	32
2		28	32
3			31
4	16	29	32
5		27	30
6			32
7	16	26	31
8		26	31
9			31

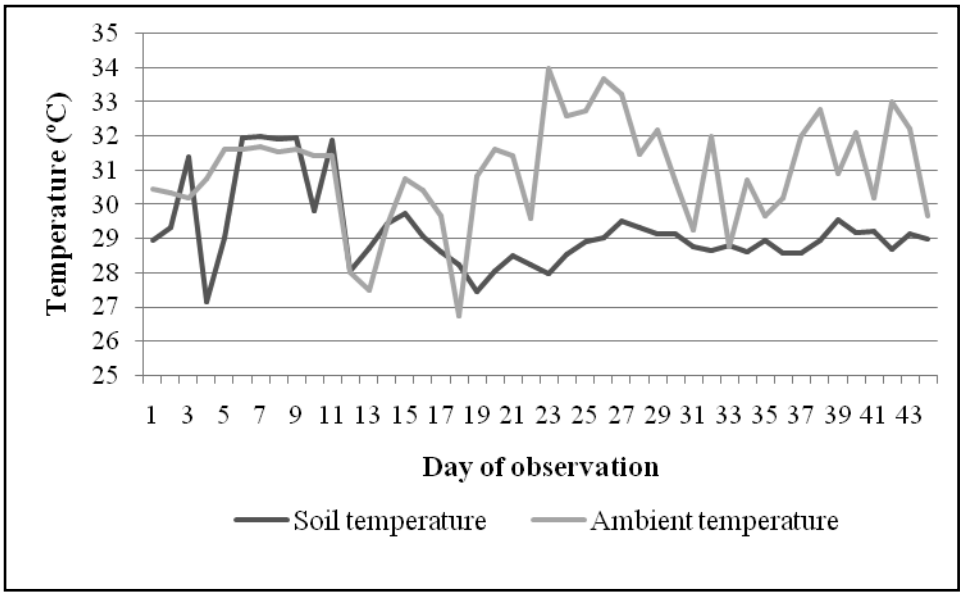

FIG. 2: Comparison of ambient temperature and soil temperature.

Table 2. For soil moisture test, control subjects started with 11.24–23.12% and then increased to value range of 25.21–26.27% as shown in Figure 4. However, the change was not consistent and the values dropped twice. For test subjects, all soil samples showed increase in moisture percentage value as shown in Table 2 and at a higher magnitude of increase compared to control subjects. For validation, 2 soil samples from each type of clothing were chosen for soil pH and moisture analysis with the same procedures used above. A ratio of 5 out of 6 soil samples could be estimated correctly based on soil pH and soil moisture percentage values indicating an accuracy of validation of 83.33%.

DISCUSSION

Generally head, neck and limbs of all subjects decomposed faster than the body trunk. The body trunk of subject KX underwent mummification because the skin was directly exposed to the atmosphere due to absence of clothing. Mummification has been shown to occur at high

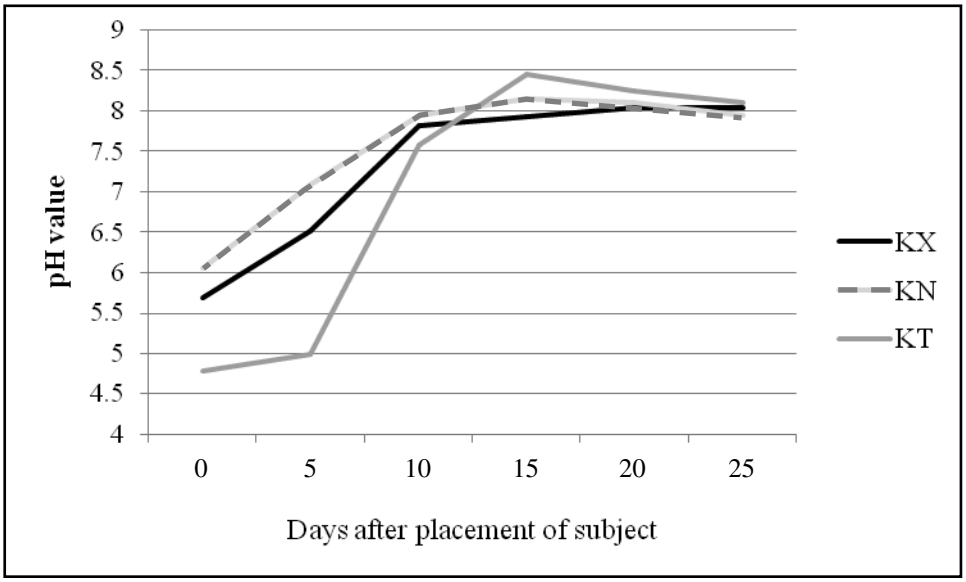

FIG. 3: Change of soil pH value in control subjects.

TABLE 2: Change of soil pH value in test subjects

Subject	pH value before burial	pH value after burial	Difference	Moisture percentage before burial (%)	Moisture percentage after burial (%)	Difference
1	5.247	6.647	1.400	19.2334	21.5695	2.3361
2	5.690	6.983	1.293	18.0763	23.3655	5.2892
3	4.957	8.313	3.356	22.3503	26.1420	3.7917
4	5.097	6.173	1.076	23.7966	25.9962	2.1996
5	5.807	6.983	1.176	18.7043	23.5785	4.8742
6	4.917	7.373	2.456	23.3038	25.8204	2.5166
7	5.187	5.693	0.506	22.5825	28.3573	5.7748
8	5.750	8.433	2.683	19.4900	22.7991	3.3066
9	4.867	8.297	3.430	21.7821	26.0116	4.2295

or low temperature as long as the air humidity is low.[18] Mummification of rabbit carcasses are more reflective of the decomposition of newborns in the real forensic context - they tend to mummify compared to adults because they are relatively sterile.[19]

Adipocere was found on subjects 4, 5 and 7 due to the bacterial partial degradation of lipid.[6] Its formation requires wet, anaerobic conditions at temperatures between 22–40ºC causing it to harden with time or when free fatty acids produced by bacteria interact with ions such as calcium and magnesium.[5] The formation of adipocere and mummification in this study is similar to the findings of Schotsmans[5] in which bodies buried in shallow graves may form adipocere while body parts that are exposed to the atmosphere will mummify. Development of TBS score in this study was faster compared to the study done by Bachmann and Simmons. They buried clothed and unclothed rabbits in shallow graves for 50 days and the TBS score recorded was 20–26[7] while TBS score in this study already exceeded 30 on day 43 after burial. This shows that the decomposition process in Malaysia is faster than Western countries, including bodies buried in shallow graves.[15] This is mainly because the warm weather and relatively high air humidity of the tropical climate in Malaysia provide an ideal setting for the decomposition process to progress rapidly as compared to temperate climate countries.

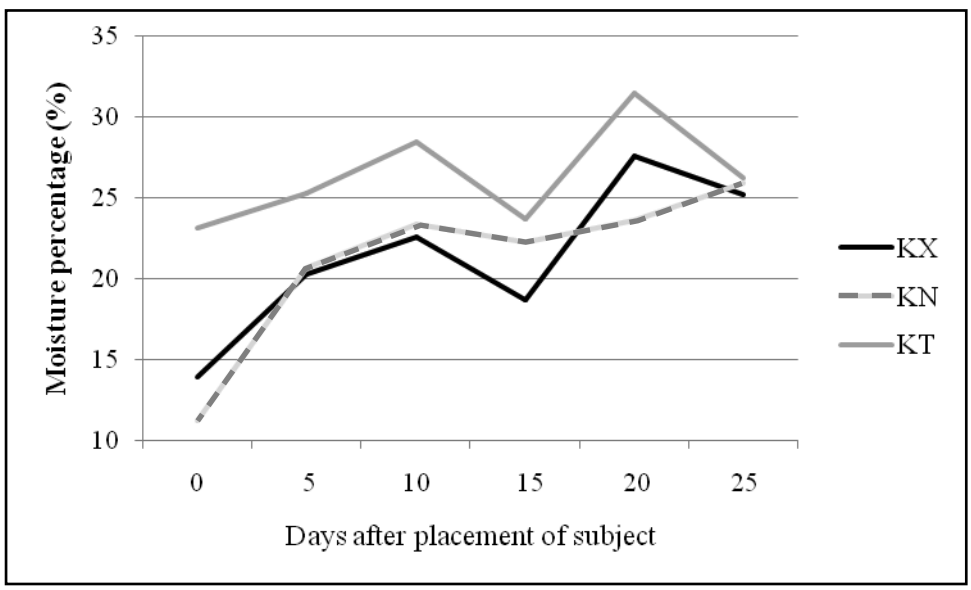

FIG. 4: Change of soil moisture percentage in control subjects.

According to Myburghs' study, the 3 main factors affecting the decomposition process are insect access, temperature and burial factor.[18] In this study, burial slowed down decomposition because the insect activity was low, especially maggot activity which is important for consumption of soft tissues. The soil layer blocked the majority of the insect species in faunal succession. If the carcasses were exposed to pre-burial insect activity, the decay may occur faster but may still be slower compared to bodies on ground surface. This is because the soil layer still limits the accessibility of insects in subsequent waves of faunal succession.[7] In this study, maggot activity was only recorded at exhumation 2 weeks after burial and was not as high as in control subjects. This is similar to other findings that no true larvae mass was found on bodies exhumed from shallow graves.[7]

Soil temperature is lower than ambient temperature because of time taken to transfer heat from solar radiation from the ground surface to the soil.[12] Meanwhile, the Earth has its own heat capacity which causes the fluctuation of soil temperature in graves to be less pronounced. Due to low insect activity, decomposition of buried bodies mainly depend on microorganism activity and this largely relies on the surrounding temperature.[20] Hence, lower soil temperature leads to lower microbial activity and thus slower decomposition.

During the late phase of this project, a type of white fungus colony was found on all 3 control subjects' skeletons. This is similar to research from Carter and his colleagues that concluded fungus colony normally appears during the late decomposition process and consists of zygomycetes, deuteromycetes and ascomycetes, when the fungal colonies respond to concentrations of ammonium and nitrate in surrounding soil.[21]

The type of clothing was not found to be significant in affecting decomposition but variances of post mortem changes were observed. The body trunk of KX was mummified because there was no clothing to prevent evaporation of decompositional fluid. Clothing is important in retaining moisture on the subject and this is similar to findings from previous studies.[10,13,22] However, there are also contradicting studies that showed that cotton clothing causes dehydration of tissue.[5,23,24] This may be due to the variety in clothing composition and manufacturing methods hence different moisture absorption and retention ability for same types of fabric.

Myburgh explained that thin clothing can protect maggots from harmful weather conditions.[18] Some studies also showed that the presence of light clothing maintains the tissue in moist condition which favours the formation of larger size of larvae mass.[10] The same clothing protection effect was also found on subject KT which recorded the longest maggot activity and allowed some post-feeding larvae to transform into pupa. For test subjects, the clothing groups showed delay in decomposition 2 weeks post burial interval. This finding is similar to studies that showed that clothing functions as layers delaying access of arthropods in soil and also separating the body from the soil physically.[2,23]

In this study, higher sun exposure enhanced decay of the carcass and this is similar to several previous studies.[25] This is mainly because sunlight increases the temperature of the carcass thus increasing microbial effect in putrefaction.[26] Scavenger animal disturbance occurred in control subject KN. The disturbance reflects real life because scavenger animal activity is one of the decomposing factors which may occur in real forensic context if the body is disposed unprotected in an outdoor environment. Repetitive exhumation from graves also showed little increasing effect to the rate of decomposition. This contradicts most studies that found investigative disturbance delayed weight loss or decomposition.[16, 27-29] This can be explained by the methodology used in previous studies where subjects were exposed for around 30 minutes on ground surface during exhumation for post mortem change observation. This caused the bodies to be exposed to higher degree of insect activity compared to those undisturbed.

Soil samples from all locations showed an increase in pH value. The peak of increase was similar to results from previous studies.[26, 30, 31] The increase is due to ammonium released where microbes metabolize protein into nitrogenous compounds. Cations such as sodium, potassium, calcium and magnesium released from tissue may undergo mineralization and cause increase of soil pH.[31] The slight decrease of pH value can be explained by action of chemautotrophic archebacteria that oxidize ammonium into nitrate through enzymatic reaction.[32] Release of other organic decompositional products such as acetic acid, phenolic compound and fatty acids may also increase the acidity of soil.

Samples from all locations showed increase in soil moisture percentage due to high amount

of decompositional fluid released. The change of values however, was inconsistent and this could be caused by weather effect including rainfall and sunlight. Increase of soil moisture content was higher in test subjects than control subjects because of higher retention of moisture in shallow graves. Particle size of soil and its moisture-absorbing characteristic tend to form "water trap" in shallow graves and prevent the fluid from dispersing into the surroundings.[13]

As for limitation of this study, the sample size was not large enough to statistically prove the effect of type of clothing on decomposition rate. The observation interval was also too wide thus capturing less data input for statistical analysis. Bigger sample size with shorter observation interval could be applied in future studies especially for buried subjects so that the development of TBS score and decomposition process in shallow grave could be studied in better detail. The period of study should also be expanded to allow all subjects to reach full TBS score. Alternative animal species can be used such as monkey (*Macaca fascicularis*) and domestic pig (*Sus scrofa*) because of better resemblance to the human body anatomically and physiologically. Soil moisture percentage analysis was influenced by the weather condition until the trend of value change was not found. To overcome this, indoor experimental setting could be used so that any influence of this factor can be avoided. Further research about the role of microorganism in putrefactive process underground and its taphonomic significance should be conducted for better understanding of decomposition process.

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