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Research Article

The Influence of Carcass Microlocation on the Speed of Postmortem Changes and Carcass Decomposition [1]

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Abstract

Determining the post-mortem interval (PMI) is often a very demanding and delicate job which requires a good knowledge of postmortem changes. In this study, 20 domestic pig carcasses *(Sus scrofa)* whose death occurred within 8 h before the start of the study were simultaneously laid at the same geographical location, but in different environments (on the ground surface - S; buried in the ground - G; placed in a crate and buried in the ground - C; submerged in water - W; and hanging in the air - A). One carcass from each group was sampled on days 14, 28, 120 and 190 from the beginning of the experiment, and on that occasion, a detailed analysis of postmortem changes and an autopsy was carried out. The difference in the rate of decomposition among groups was statistically significant. The fastest decomposition occurred in carcasses placed in a crate and buried, because during the winter period the temperature in the air was below 0°C. At that time, the decomposition process and the insect activity were slowed or stopped on carcasses in groups S, A, W, and G to some extent, while the ground and wooden crate were good thermal insulators for group C carcasses and provided better conditions for insect activity.

Keywords: *Forensic veterinary medicine, Postmortem changes, Decomposition, Taphonomy, Pig carcass*

Karkas Mikrolokasyonunun Postmortem Değişiklikler ve Karkas Dekompozisyonu Üzerine Etkisi

Öz

Ölüm zamanını belirlemek çoğu zaman zor ve hassas bir iş olup postmortem oluşan değişiklikleri bilmeyi gerektirir. Bu çalışmada ölümleri 8 saat içerisinde gerçekleşmiş 20 adet evcil domuz *(Sus scrofa)* karkası aynı zamanda ve aynı coğrafik lokasyonda fakat farklı ortamlarda (toprak yüzeyinde (S), toprağa gömülü (G), tabut içerisinde toprağa gömülü (C), su içerisinde (W), havada asılı (A)) tutuldu. Her gruptan bir karkas çalışmanın başlandıcından sonra 14, 28, 120 ve 190. günlerde alınarak postmortem değişiklikleri belirlemek amacıyla analiz edildi ve otopsileri gerçekleştirildi. Gruplar arasındaki dekompozisyon oran farkları istatistiksel olarak anlamlı bulundu. En hızlı dekompozisyon, kış süresince hava sıcaklığının 0°C altında olması sebebiyle tabuta yerleştirilerek gömülenlerde oluştu. Bu sürede dekompozisyon süreci ve böcek aktivitesi S, A, W ve G gruplarındaki karkaslarda bir seviyeye kadar yavaşladı veya durdu. Toprak ve tahta tabut iyi bir ısı yalıtımı görevi görerek grup C'deki karkaslarda böcek aktivitesi için daha uygun şartlar sağladı.

Anahtar sözcükler: *Adli veteriner hekimlik, Postmortem değişiklikler, Dekompozisyon, Tafonomi, Domuz karkası*

INTRODUCTION

Finding, discovering and assessing the postmortem age of a corpse is always a challenge, because in order for a case to be resolved correctly it is necessary for forensic experts to first determine the time of death, i.e., the postmortem interval (PMI). The establishment of PMI is an important part of medical and legal research, aiding the team to include/exclude suspects [1,2]. The science of PMI also underpins the fundamental ability to identify a corpse in the first place [1,2]. This is a demanding task because many biotic and abiotic factors affect the decomposition of a corpse, including processes within the body itself (autolysis, putrefaction, decay). The decomposition of a corpse is a continuous

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process that begins from the moment of death and ends when only the skeleton of the corpse remains, which also decomposes, but much more slowly than other tissues. Understanding the decomposition process is the basis for a successful PMI assessment [3]. In corpse decomposition studies, pig carcasses are most often used for determining postmortem changes due to their similarities with the anatomy, physiology, and the microbiota of the human digestive tract [4,5].

In order for postmortem changes on the carcasses to be compared and used for determining PMI, total body score (TBS) is used to quantify changes in carcass decomposition. Many authors have studied the relationship between TBS and accumulated degree days (ADD) with the aim of making a more precise and more reliable determination of PMI [1,6-8]. Many authors have examined environmental influences such as temperature and humidity, the impact of physical-chemical agents, the presence of clothing on the body, and the role of insects on the speed of post-mortem changes to determine PMI [9-11]. Casper's rule states: at a tolerably similar average temperature, the same post-mortem changes develop in suspended carcasses in one week (or a month), in submerged carcasses in two weeks (or months) and in carcasses buried in the ground in eight weeks (months) [12,13]. It is still widely used [14] which illustrates the lack of suitable methods [15]. Characteristics of decomposition can be identified and distinguished, but it is almost impossible to determine the exact time of death on the basis of this data only.

As a significant amount of time has passed since this discovery was made, and every region of the world has its own climatic characteristics, the need has arisen for this research to be conducted in Serbia for the first time. Serbia is characterized by a high suicide rate [16], as well as a large number of migrants who have been passing through the country on the road to EU countries in recent years. Migrants often enter and pass through Serbia illegally, so they are not on any official records and nor are their disappearances reported. Only with the discovery of a corpse does the identification and examination of all the circumstances leading to the death begin.

The aim of this study was to describe and compare the decomposition rate of pig carcasses with the same PMI, in the same geographical location, but placed in different environments (on the ground surface, buried in the ground with and without a crate, submerged in water and hanging in the air). Also, the influence of microlocation (where a body would decompose most rapidly in the climatic conditions present during the study) on the decomposition of the carcass was determined.

MATERIAL and METHODS

This study was conducted from October 2016 until April 2017 in Serbia, in a region remote from a populated area (45°21'55.68"N 19°47'22.63"E). The climate in this region is continental, characterized by four seasons, with hot summers (temperatures up to 40°C) and cold winters (temperatures down to -20°C), while spring and autumn are mild. Rainfall mainly occurs during spring and autumn months (with average 500 mm of rainfall per annum), and the vegetation is typical for lowland areas with low vegetation.

The animals were selected from 8 farms with a population of 200.000 pigs, which were from the same epizootiological area, which means that they had almost identical living conditions [17] and approximately 50-70 pigs died during every day. Only pigs that died of natural causes, with no external wounds, were used. The criteria for selecting the 20 domestic pig carcasses *(Sus scrofa)* were that the pigs did not have clinical symptoms of disease at the time of their death, that anamnesis stated they were in good health, that they were in good body condition and that not more than 8 h had passed since the moment of death. The age of the pigs before death was 3-4 months and the carcasses weighed between 33 and 38 kg and all were in same gender (female).

Four pig carcasses were placed in each of five situations: on the ground surface (S), buried in the ground (G), in a wooden crate and buried in the ground (C), submerged in water in a barrel (W), or hanging in the air (A). The experiment was simultaneously set up for all five groups. Four pig carcasses were placed on the ground surface; carcasses were under one protective net of wire and sheet metal, in order to be protected from predators. Four pig carcasses were each buried in a separate 120 cm deep hole in the ground ($CaCO_3$ 15.52, N 0.1, P_2O_2 23.63, K_2O 27.59, pH 7.31). The distance between each of the holes where the carcasses were placed was 5 m. Four pig carcasses were placed in four separate wooden crates measuring 120 x 45 x 40 cm. The wooden crates were buried in the ground at a depth of 120 cm, each 5 m apart. Drinking water (Ca^{2+} 75.2, Mg^{2+} 14.5, K^+ 1.25, Na^+ 10.0, HCO_3^- 307.1, Cl^- 2.3, SO_4^{2-} 7.6, pH 7.6) was placed in four 300 l barrels, and one pig carcass was placed in each barrel. This part of the study was performed in a place protected from direct sunlight and surrounded by trees and overgrown grass. Finally, four pig carcasses were hung with a rope around the neck area on a metal tube placed between two trees. The distance between the carcasses was 80 cm, and the distance between the hind legs of the carcasses and the ground was 1.5 m. Also, hanging carcasses were protected from direct sunlight and surrounded by trees.

The carcasses were examined and measured before the study began. From all the groups (S, G, C, W and A), one carcass was taken on days 14, 28, 120 and 190 from the beginning of the study. The carcasses were transported to an autopsy room where a detailed examination of the carcasses took place with weight measurements and autopsies. All characteristic changes were descriptively described and photographed. TBS was used to assess the rate of decomposition, and the qualitative descriptions of the stages of decomposition were converted into quantitative scores.

TBS to quantify postmortem changes was expressed in points depending on the decomposition rate, and has been previously described [7,8]. The head and neck (0-12), trunk (0-11) and limbs (0-9) were separately scored. For all three body regions, point 0 represented a fresh carcass without discoloration, and the last point was the stage of skeletonization.

Temperature and precipitation was measured throughout the study and the daily maximum, minimum and average temperatures were recorded. For evaluation of PMI, ADD and TBS were used. To measure the temperature, a data logger located on site was used, as was data from the Republic Hydrometeorological Institute, which has a station 1.8 km from the experimental site. ADD was calculated according to the formula ADD=(Tmax+Tmin)/2-threshold, where Tmax is the maximum daily temperature, Tmin is the minimum daily temperature, and 0°C was taken as the threshold, a value that is accepted in many forensic studies [18-20]. Starting from the first day, the daily ADD obtained was cumulatively added for each day, and when Tmax+Tmin<0, 0 was added for that day.

The two-way ANOVA was conducted, where TBS was selected as the dependent variable, and the body region, microlocation and day were selected as independent factors. Estimated influence of factors and their interaction (body region×microlocation, body region×day and micro-location×day) to TBS.

RESULTS

Meteorological Data

The geographic location where the research was conducted has a characteristic continental climate with four seasons. The study lasted from mid-autumn to mid-spring. The minimum and maximum temperature, as well as the amount of precipitation, was recorded daily *(Fig. 1)*. The highest temperature during the study was 26.2°C (April) and the lowest was -19.4°C (January). There was a total of 29 days when the temperature did not exceed 0°C within a 24 h period. During the study, a total of 176.3 mm/m^2 of rain fell, and snow fell for 18 days while snow cover lasted for 33 days.

Weight of Pig Carcasses

After 14 days, the weight of carcasses in groups S, G, C, and A was reduced while the weight increased in group W. Similarly, on days 28 and 120, the weight of carcasses in groups S, G, C and A continued to decrease almost linearly while the weight of the carcasses in the water remained virtually unchanged compared to their weights on day 14. The weights of carcasses from groups S, G, C and W were not measured at the end of the study, on day 190, because they were in an advanced stage of decomposition (or total skeletonization) and it was not possible to take weight measurements. Only the weight of carcass A was measured on day 190, and it was 35.4% of the carcass weight measured at the beginning of the study *(Fig. 2)*.

Decomposition Process of Pig Carcasses

As pig carcasses in groups G and C were buried, and group W submerged in water, changes were noticed only after carcass exhumation or removal from water. Comparable postmortem changes during the decomposition of carcasses are described in *Table 1*. During each sampling, the decomposition stages of the carcasses was assessed and the TBS for each carcass was determined *(Table 1)*. *Fig. 3* shows decomposition stages of carcasses from the different environments immediately prior to sampling and autopsies.

At the first sampling on day 14, the carcasses from all groups were approximately in the same decomposition

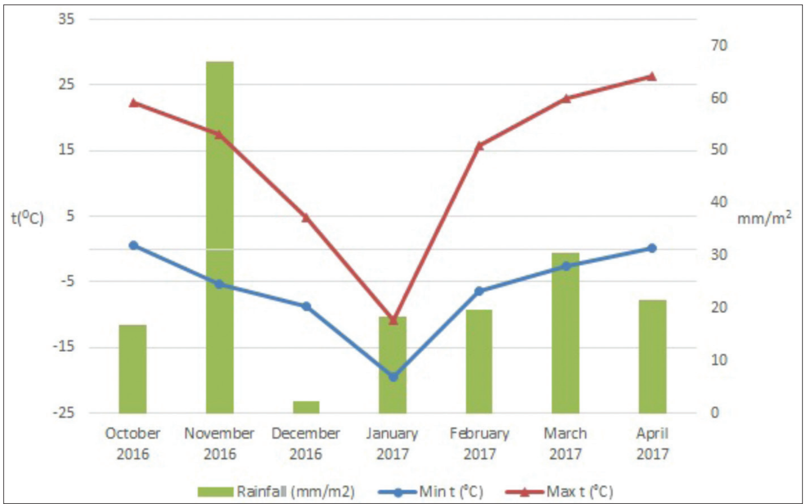

Fig 1. The minimum and maximum temperatures with precipitation data during the study period (October 2016 to April 2017)

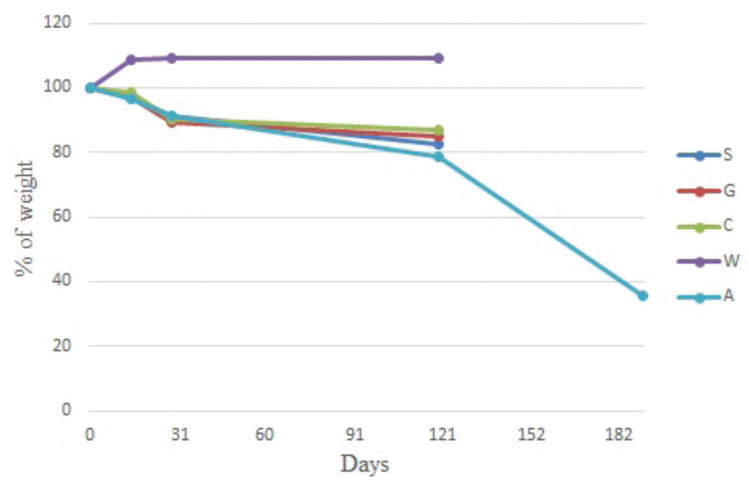

Fig 2. Weight of pig carcasses at the beginning of the experiment and the moment of the sampling

Table 1. *Summary of postmortem interval, accumulated degree days and total body score for three body regions in carcasses from different microlocations and decomposition stage at the moment of carcass sampling*

PMI	ADD	Body Regions	S	G	C	W	A
14	127.8	HN	1	1	1	1	2
		T	2	1	1	1	2
		L	1	1	1	1	1
		TBS	4	3	3	3	5
		Decomposition stage	Bloat	Bloat	Bloat	Bloat	Bloat
28	224.3	HN	2	2	3	2	8
		T	2	1	3	2	5
		L	2	2	3	2	4
		TBS	6	5	9	6	17
		Decomposition stage	Bloat	Bloat	Active decay	Bloat	Active decay
120	414.1	HN	4	4	7	4	8
		T	4	4	5	4	7
		L	3	4	5	3	5
		TBS	11	12	17	11	20
		Decomposition stage	Active decay	Active decay	Advance decay	Active decay	Active decay
190	1130.6	HN	10	8	12	7	10
		T	8	6	11	10	9
		L	8	6	9	7	7
		TBS	26	20	32	24	26
		Decomposition stage	Advance decay	Advance decay	Skeletoni-zation	Advance decay	Advance decay

PMI-postmortem interval; ADD-accumulated degree days; S-on the ground surface; G-buried in the ground; C-in a wooden crate and buried in the ground; W-submerged in water in a barrel; A-hanging in the air; HN-head and neck; T-trunk; L-limbs; TBS- total body score

stage (TBS scores of 3-5). On day 28, there was a significant difference in the decomposition of carcasses in the experimental groups. Carcasses S, G, and W reached approximately the same decomposition stage (TBS of 5-6), while the carcasses in the crates (C) decomposed much faster reaching TBS of 9, while the hanging carcasses (A) were in the fastest reaching TBS of 17 in the same period. On day 120, the ratio of the decomposition stages was similar, as carcasses in groups S, G and W were still at approximately the same decomposition stage (TBS of 11-12) while carcasses in groups C and A were at a faster reaching decomposition stage (TBS of 17 and 20, respectively). By day 120, decomposition was fastest on hanging carcasses and then, during the period from days 120 to 190, the fastest changes took place on the carcasses buried in the crate. At the end of the study, (after 190 days) total skeletonization had occurred only in the group C carcass (TBS of 32), while the lowest decomposition stage (TBS of 20) was established in the carcass buried in the ground (G), and the remaining three groups (carcasses S,

Fig 3. Comparison of decomposition process of the carcasses from groups S, G, C, W and A. 0, 28 and 190 days

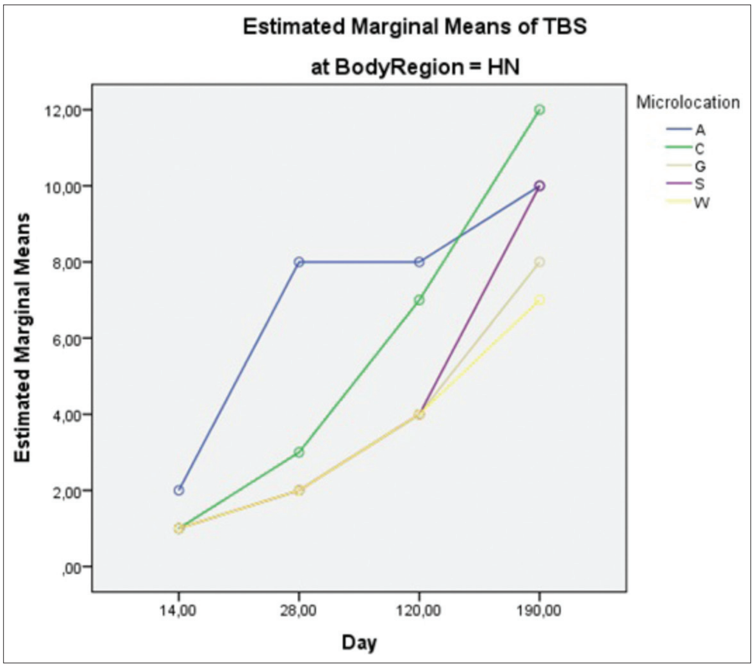

Fig 4. Graph showing the influence of head and neck, day, microlocation and their interaction to total body score

W and A) were at equal stages of decomposition (TBS of 24-26) *(Table 1* and *Fig. 3)*.

The speed of postmortem changes and carcass decomposition indicate influence of body region (F=6.364, P<0.05), microlocation (F=4.279, P<0.05) and day (F=45.642, P<0.001) to TBS, and their interaction differences were observed between body region×microlocation (F=2.789, P<0.05) and microlocation×day (F=5.842, P<0.001), while between body region×day no significant difference was observed (F=2.023, P>0.05). Decomposition of body regions, head and neck (HN), trunk (T) and limbs (L) was statistically different among groups (S, G, C, W, A) during the study *(Fig. 4-6)*.

DISCUSSION

Many authors have examined PMI in relation to various environmental influences such as temperature, freezing, soil, insects, lime, body size, or clothing [3,21-24]. As temperature is the leading external factor for the decomposition of a carcass, when determining PMI, the most common temperature influence is expressed through accumulated degree days (ADD) [25,26]. In this research, all carcasses had the same PMI, which was determined at the beginning of the study, and all carcasses were positioned at the same geographic location at the same time, and therefore, were exposed to the same general atmospheric conditions, but in different environments (on the ground, in the ground, in a crate in the ground, in water or hanging in the air). A comparison of the decomposition rate over the same time interval indicates the influence of the microlocation of the body on the speed of the development of post-mortem changes.

The weight or the size of the carcass affects the rate of postmortem changes and the PMI, and it is known that smaller carcasses decompose faster than larger ones [21]. The carcasses in all experimental groups were of approximately the same size and, therefore, the influence of the size of the carcasses in the different experimental groups on the decomposition rates was negligible. Only the carcass in the water (W) underwent an increase in weight (9%) on day 14 of the study, which was due to the passive entry of water into the respiratory and digestive tract organs. The same increase in weight was found on days 28

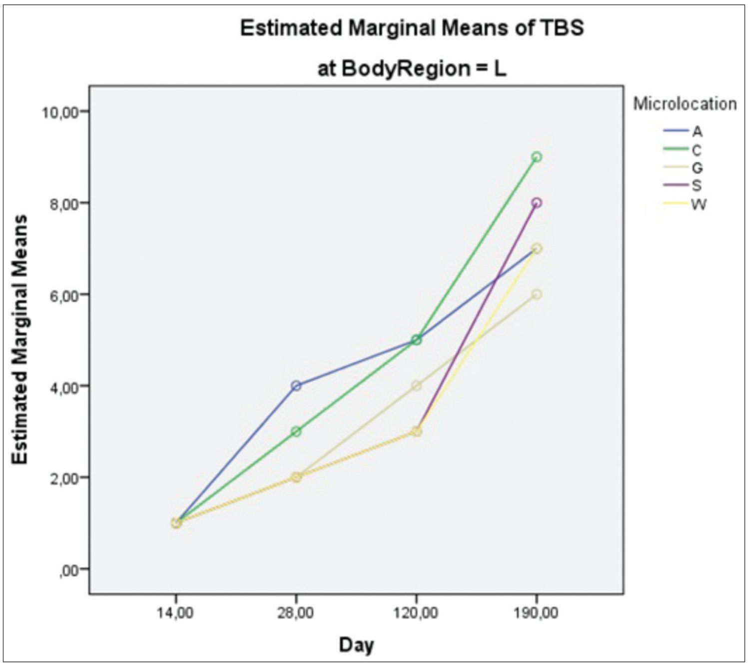

Fig 5. Graph showing the influence of trunk, day, microlocation and their interaction to total body score

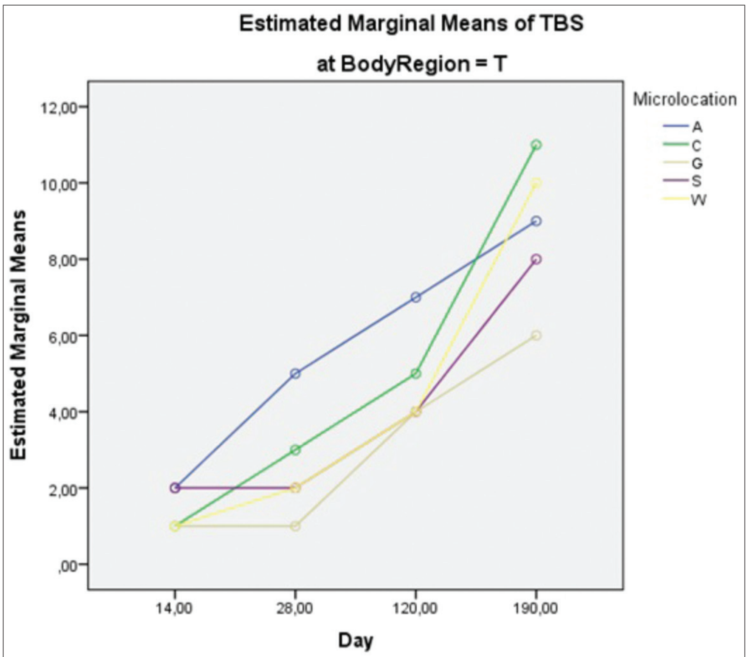

Fig 6. Graph showing the nfluence of limbs, day, microlocation and their interaction to total body score

and 120. Group A carcasses underwent the greatest weight loss, and on days 120 and 190 had lost 21.2% and 64.6%, respectively, of the weight measured at the beginning of the study. Although on day 190 only the carcass from group A was compact and could be measured, the small weight observed was due to the high degree of dehydration and mummification. Weight loss which occurred in carcasses from groups S, G, C, and A was accompanied by the decomposition we observed, and our finding the weight decreased has been confirmed by other studies [25,26]. However, proof that this cannot be relied on with any certainty to determine the PMI is the weight of group W carcasses as well as the possibility of losing certain body parts as a result of scavenging or in the case of the hanging pigs, as a result of skeletal element loss via gravity [22].

Since all carcasses were situated at the same geographic location and were exposed to the same general atmospheric conditions at the time of sampling, they would have had the same ADD if only the air temperature were taken into account *(Table 1)*. However, the temperatures to which the carcasses from the groups were actually exposed were different (air, water, earth temperature), and hence, a comparison of changes depending on ADD would have inaccuracies. As the PMI in this research was predetermined and known, with the aim being to compare the same PMI for carcasses set in different environments, the ADD at an internal level for each group was not required, but the ADD values from this study *(Table 1)* can be used for comparison with similar studies by other authors.

Until day 120, carcasses from groups S and G were in very similar decomposition stages, as determined in previous studies [1,7,27], while after day 120, an increase in temperature *(Fig. 1)* quickened the rate of decomposition of carcasses on the ground surface [28,29]. Rainfall did not necessarily cause an increase in decomposition, because the largest quantity of rain fell in the first 28 days of the study *(Fig. 1)*, and there was no difference in the decomposition of the carcasses exposed to the rain (group S) and those sheltered from the rain (group G). After day 120, the slower decomposition of carcasses buried in the ground was the result of lower temperatures in the ground than on the ground surface and in the air and because that reduced exposure to insects. Troutman et al.[30] found that carcass decomposition may be up to eight times slower in the ground compared to on the ground. This could not be confirmed in our study, as carcasses in groups S and G did not reach skeletonization stage on day 190. As in the study carried out by Marais-Werner et al.[7], carcasses in group S and G were in the decomposition stage of TBS <26 on day 190.

During the early stages of decomposition, group A carcasses experienced the highest rate of postmortem changes and decomposition due to the carcasses' exposure to insects, and to disturbances to the position of the abdominal cavity organs as a result of the effects of the ground and atmospheric phenomena such as wind and humidity. Group S carcasses were exposed to very similar conditions, except that they were on the ground, which resulted in the same degree of decomposition in both groups at the end of this study. Other authors have reached similar results [22,26] in their studies, with the exception that the early stages of decomposition occurred earlier on carcasses on the ground surface.

Between day 28 and day 120, i.e., during the winter period, when the lowest temperature occurred *(Fig. 1)*, the ADD increased very slightly (from 224.3 to 414.1), which resulted in the cessation of insect activity, and thus, in the decomposition of the carcasses in groups S, G, A and W. The greatest degree of decomposition among all the different groups of carcasses occurred in group C as a result of the influence of insect activity and temperature, because ground at the depth of 120 cm and wooden crate were good thermal insulators [15], and there were 29 days when the temperature was 0°C, and then, the decomposition processes in the other groups of carcasses was stopped. Although group C carcasses were in a crate and buried in the ground at a depth of 120 cm, the presence of a large number of larvae was noticed on day 120 and day 190. Although it is not common to find insects on the carcasses in the ground at that depth, it is possible this is a consequence of the contamination of the corpses and/or wooden crates during the period from death to burying, because in that period the daily temperatures were 20°C and higher. Also, larvae presence can be a result of higher temperatures in the wooden crate than in the air during the winter period, and therefore, more favorable conditions for insect activity [11,31]. That and the looseness of the ground could have enabled easy passage of insects to carcasses. After 120 days, temperatures began to rise and ADD increased from 120 to 190 days (from 414.1 to 1130.6), resulting in both insect activity and faster decomposition of the carcasses in the other groups.

On carcasses submerged in water, the fastest changes occurred on the trunk which appeared above water during active decay stages despite suggestions in the literature that the head always decomposes at a faster rate than the body. Previous studies by other authors [32,33] showed that the decomposition of soft body tissues occurred at the same rate both in the water and on the ground surface, which was confirmed in this study. Certainly, the decomposition rate of a carcass in naturally occurring waters will differ from the decomposition rate of carcasses in this study as decomposition is influenced by a series of water characteristics, with its specific flora and fauna, as well as the depth at which the carcass is located [28].

Comparing the decomposition of carcasses in this study with Casper's rule, we have almost the opposite finding, i.e. in this study, the carcass placed in a wooden crate in the ground was the first to decompose, while the times for the decomposition of the carcasses left in the open or in water were almost identical. Some authors [34] have explained Casper's rule by different quantities of oxygen in the air or water, i.e. the differences in the rates of putrefactive changes are based on the dissimilar amount of oxygen in the air, water and underground, which was not confirmed in this study.

The present study has found difference in the rate of decomposition among groups. Decomposition rates were significantly different in the different body regions (HN, T and L) of the carcasses at different microlocations *(Fig. 4-6)*, due to the characteristics of the environment, i.e the microlocation on/in which the carcasses had been laid.

In this study, temperature had the greatest impact on decomposition directly and indirectly through insect activity. In the period from November to March, the average minimum temperature was below 0°C *(Fig. 1)*, which slowed or stopped the decomposition processes for the carcasses that were left outdoors (S, A, W, and G to some extent). Although it was physically harder for insects to reach the carcasses in the ground (G and C), the influence of ground and wooden crate as thermo-insulators led to higher temperatures at these microlocations and better conditions for insect activity, which resulted in faster decomposition of the carcasses. Water also presented a physical barrier for insects, so the decomposition was the fastest on the part of the carcass (trunk) that was out of the water and exposed to a much higher oxygen level [34], and consequently more accessible to insects *(Fig. 3)*.

In this research, after the early stage of decomposition, the fastest development of postmortem changes and decomposition occurred on carcasses buried in a crate underground. All carcasses had the same PMI and were laid at the same geographic location, but in different environments (on the ground, in the ground, in a crate in the ground, in water and hung in the air), which had a crucial impact on the decomposition rate. The discovery of two or more carcasses at the same geographical location does not automatically mean that they must have the same PMI, but rather, it is necessary to pay special attention to an analysis of the microlocation in which the carcass is located. These results should be useful as a key for determining PMI and comparing PMI between corpses found at the same time in the same location, but in different environments. Certainly, this is the first study of this kind to be conducted in Serbia and the region. However, this topic is deserving of further research due to the geographical position of the Balkans and current and historical events related to this area, because of which there is a need to improve existing databases for the determination of PMI.

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