Assessment of Entomological Remains from Soil Samples Collected from a Pig (*Sus scrofa domestica*) Carcass Decomposition Site after 13 Years

On Üç Yıl Sonra Domuz Leşinin (Sus scrofa domestica) Dekompozisyon Bölgesinden Toplanan Toprak Örneklerindeki Entomolojik Kalıntıların Değerlendirilmesi

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ABSTRACT

Objective: Carrion insects inhabiting the soil play an important role in forensic investigations because they may help to solve both active and cold cases. The aim of this study was to examine the entomofauna of forensic importance in soil samples removed after 13 years from a pig carrion decomposition site.

Methods: Soil samples were collected from an old carrion decomposition study site in Bâla, the Ankara Province. Four holes, approximately 40 cm deep and 35 cm width were excavated at the study site. The samples were collected and placed in ventilated cups. Each cup was labeled mentioning the excavation location, time, date, and name of the collector. Insects and their remains found in the soil were collected by sweeping the soil from the specimens using a brush. The insects were morphologically identified.

Results: A total of 635 specimens of Calliphoridae, Dermestidae, Cleridae, Staphylinidae, Histeridae, and Formicidae were identified. Flies such as *Chrysomya albiceps* (Wiedmann, 1819), and beetles such as *Dermestes frischii* (Kugelan, 1792), Necrobia rufipes (De Geer, 1775), and *Creophilus maxillosus* (Linnaeus, 1758), were identified as the species.

Conclusion: Our results show that soil samples still harbor entomological specimens after 13 years. This study, to the best of our knowledge, was the first of its kind in Turkey. Forensically, important insects and their remains may be identified in the soil long time after the corpse is buried. Consequently, cold cases may be solved using insects.

Keywords: Carcass, decomposition, entomofauna, forensic entomology, soil

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ÖZ

Amaç: Toprakta yaşayan böcekler, aktif ve eski olguların çözülmesine yardımcı olabileceğinden, adli araştırmalarda önemli rol oynamaktadır. Bu çalışmanın amacı, domuzun ölümünden 13 yıl sonra toprak örneklerinden çıkartılan adli önemi olan entomofaunayı incelemektir.

Yöntemler: Ankara ili Bâla ilçesinde on üç yıl önce ölen bir domuz leşi dekompozisyon çalışması alanından toprak örnekleri toplandı. Çalışma alanında yaklaşık 40 cm derinliğinde 35 cm genişliğinde 4 geniş delik kazıldı. Numuneler toplandı ve havalandırmalı bardaklara yerleştirildi. Her bardağın üzerine yer, saat, tarih ve toplayanın adı yazıldı. Topraktaki böcekler ve kalıntıları mikroskop altında toprağın bir fırça yardımıyla süpürülmesi ile toplandı ve morfolojik olarak tür tayini yapıldı.

Bulgular: Calliphoridae, Dermestidae, Cleridae, Staphylinidae, Histeridae ve Formicidae familyasına ait toplam 635 örnek tespit edildi. Sineklerden *Chrysomya albiceps* (Wiedmann, 1819), ve kınkanatlılardan ise *Dermestes frischii* (Kugelan, 1792), *Necrobia rufipes* (De Geer, 1775) ile *Creophilus maxillosus* (Linnaeus, 1758) türleri teşhis edildi.

Sonuç: Bu bulgular, on üç yıl sonra hala toprak numunelerinin entomolojik örnekleri barındırdığını göstermiştir. Sunulan çalışma Türkiyede ilk kez yapılmıştır. Sonuç olarak, adli önemi olan böceklerin ve kalıntılarının, ölüm yerinin toprağından uzun süre sonra bile tespit edilebildiğini ve aynı zamanda çözülememiş eski adli vakaların da böceklerden yararlanılarak çözülebileceğini göstermiştir.

Anahtar Kelimeler: Ceset, dekomposizyon, entomofauna, adli entomoloji, toprak

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INTRODUCTION

Forensic entomology is the study of the arthropod development and colonization of corpses in the succession pattern to solve legal cases (1-3). It is commonly used to estimate the time between death and the corpse discovery. This period is called the postmortem interval (PMI) (4). When this interval is greater than 72 hours, forensic entomology methods can be more accurate in estimating the PMI than traditional medical techniques, and sometimes they are the only available techniques (4-6). After death, the odor emitted by the carcass attracts different insect species. Flies (Diptera) and beetles (Coleoptera) are the first to reach the carcass and are the most common species. Among dipteran species, blowflies (Diptera: Calliphoridae) are the initial colonizers of dead bodies (6-9). Arthropods associated with a carcass are generally classified into four ecological categories: necrophages, parasites and predators of the necrophages, omnivores, and adventive species (7). Arnaldos et al. (10) reported that the Formicidae species use the body as a shelter to obtain humidity and food and that they may be considered omnivorous. The Formicidae species belong mainly to the local fauna and may be present even before the body placement. Thus, these species are of lesser forensic importance than the necrophagous ones.

Body decomposition in a terrestrial environment alters the substrate beneath (11). This initiates a series of changes in vegetation and fauna, beginning a succession of arthropods affected by the carcass decomposition. Fluids that are a product of decomposition and associated carcass fauna are reported to change the soil to a depth of 14 cm, affecting mostly the upper layers. The soil that is directly beneath and surrounding the body represents a decompositional zone occupied by the carcass dwellers distinct from a surrounding area of approximately 10 cm. This provides an intermediate zone inhabited by both the carrion and regular soil-dwelling invertebrates (12).

Insects found on buried bodies in the soil for a short or long period are of a great forensic interest. Some arthropod species associated with decomposing bodies are not found on the remains, but are distributed around, or even inhabiting the soil under the body. It is thus possible to prove whether the dead body was moved or not after death and to determine the primary and secondary crime scenes. Therefore, it is crucial to collect soil samples during criminal investigations (12).

In this context, the aim of this study was to examine the entomofauna of forensic importance in soil samples 13 years after death.

METHODS

The Study Area

A field trial with a decomposing carcass of a pig (*Sus scrofa domestica*) was conducted in Çavuşlu, Bâla, Ankara (39°40'59.0"N 33°00'13.1"E) during the years from 2003 to 2005. In January 2016, four holes, approximately 40 cm deep were excavated at the study site. The buried pig skeleton was found. The soil samples were collected and placed in polystyrene disposable cups. Each cup was labeled mentioning the excavation location, time, and date.

The collected samples were transferred to the Forensic Sciences Institute of Ankara University and examined under a Leica S8 APO stereo zoom microscope.

Laboratory Study

The soil was dried on a sheet of paper in the laboratory. Insects and their remains found in the soil were collected by sweeping the soil from the specimens using a brush. They were then photographed (Canon PowerShot S60) (Figures 1-6) and morphologically identified using standard taxonomic keys (13, 14).

RESULTS

In this study, we describe the entomofauna of forensic interest found in the soil samples recovered from an old decomposition study's location. A total number of 635 specimens of Calliphoridae, Dermestidae, Cleridae, Staphylinidae, Histeridae, and Formicidae were found. The identification results of insect species and their numbers are presented in Table 1. We noticed that the soil samples were still harboring entomological spec-

Figure 1. Diptera Pupae. A: *Chrysomya albiceps* pupa; B: Fannidae sp. pupa.

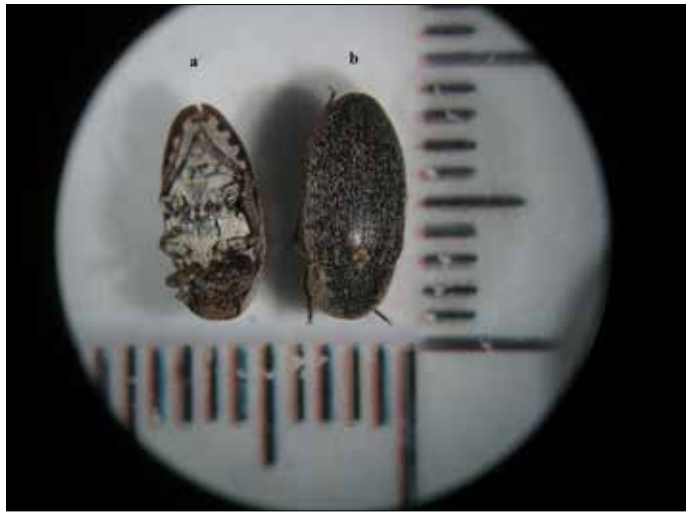

Figure 2. *Dermestes frischii* adult. A: ventral view; B: dorsal view.

Figure 3. *Dermestes sp.* larva.

Figure 5. *Creophilus maxillosus* adult.

Figure 4. *Necrobia rufipes* adult. A: dorsal view B: ventral view.

Figure 6. Coleoptera elytra.

imens after 13 years. Overall, 28.2% of the of the specimens were identified at the species level, and they included *Chrysomya albiceps* (Wiedmann, 1819), *Dermestes frischii* (Kugelan, 1792), *Necrobia rufipes* (De Geer, 1775), and *Creophilus maxillosus* (Linnaeus, 1758).

DISCUSSION

Necrobia rufipes is a slow-developing species depending on temperature. It is known to be the predator of other insects and cannibal in the presence of other species in large numbers. This predatory behavior also occurs in the third larvar instar of the *Chrysomya albiceps* species (9, 15, 16). *Creophilus maxillosus* is a scavenger and can be found on carcasses, and it is also the predator of carrion maggots (17-19). This predatory behavior is observed when the large numbers of Diptera larvae are present in the soil. *Dermestes frischii* is a species widely distributed all over the world. It is usually found in dry fish and legumes. Lefebvre and Gaudry (20) report that the dermestids arrive to animal carcasses in the third wave, during the advanced decay stage.

Carter and Tibbett (21) state that the soil is a passive environment, influenced by the surroundings. Animal carcasses and plant cover mixed into the soil cause chemical and microbiological disturbances in the soil structure. The return of the original soil structure takes a long time (21). These findings support our results as the soil preserved the specimens for years. It might not have been possible to recover the samples in case of the lack of information about the body's site. At a depth of 40 cm, some of the specimens were found buried in the soil due to rainfall and soil accumulation. Merritt et al. (22) discovered many arthropods in a grave opened 28 years after death. Some of them were reported to be Collembola (springtails), Acarina (mites) from the Glycyphagidae family, and Diptera pupae of the Phoridae family. Anderson and van Laerhoven (23) likewise stated that even 271 days after death, the vegetation and soil fauna had not returned to normal. Our results are in agreement with these findings. Based on these observations, it may be proved that a body was removed from its original location when necrophagous insects or their remains are not found in the soil samples.

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Table 1. Insect species and their remains identified from the soil samples.

Order	Family	Species	Stage/insect part	Absolute abundance	Ecological category
Diptera	Calliphoridae	*Chrysomya albiceps* (Wiedmann, 1819)	Larvae	4	Necrophagous
			Pupae	144	
Diptera remains			Heads Wings Legs Larvae Pupae Puparіums	130	
Coleoptera	Dermestidae	*Dermestes frischii* (Kugelan, 1792)	Adult	15	Saprophagous
		Dermestes sp.	Larvae	11	
	Cleridae	*Necrobia rufipes* (De Geer, 1775)	Adults	5	Saprophagous
	Staphylinidae	*Creophilus maxillosus* (Linnaeus, 1758)	Adults	11	Predator
	Histeridae	*Saprinus* sp.	Adults		Predator
Coleoptera remains			Adults parts Larvae Larval exuviates Elytra Wings Legs	305	
Hymenoptera	Formicidae	Formicidae species		5	Omnivorous
Total				630	

CONCLUSION

It is mandatory to collect soil to solve active and cold forensic cases. Insects of forensic interest and their remains can be identified from the soil long time after death. This practice may contribute greatly when determining whether a dumped body was shredded by scavenger vertebrates or removed from its original place.

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