H50 Insects and Bacteria as Forensic Decomposition Markers of Buried Rat Carcasses

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After attending this presentation, attendees will have a detailed understanding of carcasses decomposing in soil and of the necrophagous entomofauna identified during the stages of decomposition. Moreover, attendees will be provided with information regarding the bacterial dynamics of buried carcasses and with the proposal of two bacteria taxa as putative markers for the postmortem interval estimation.

This presentation will impact the forensic science community by adding important data regarding the decomposition process in soil as well as the insects and bacteria associated with carcass decomposition. This type of research, rarely studied to date and the experimental research conducted on exposed remains, will provide much-needed qualitative and quantitative information on necrophagous insects and bacteria associated with buried carcasses.

The decomposition process of surface remains and their associated necrophagous entomofauna has been well studied compared with buried carcasses. The insect diversity and succession pattern for buried and exposed carcasses are different due to the variations of edaphic and environmental conditions. In the case of buried remains, the occurrence of necrophagous insects can be delayed or inhibited by the climatic conditions, soil type, and depth of burial. At the same time, the carcass bacterial community structure is expected to differ between buried and exposed conditions. This becomes an important distinction in cases such as violent criminal actions, when the perpetrator tends to dispose of the cadaver by frequently burying it in a shallow grave.

In this context, the current study focused on monitoring the decomposition process of buried rat carcasses from shallow graves, the diversity and dynamic of insects and bacteria throughout the decomposition stages, and the environmental parameters' influence on these variations at a depth of 40cm. The survey took place in the spring (March) and summer (June) months of 2016 in a green urban area of Bucharest, Romania. For each case, 30 rat (*Rattus norvegicus*) adults were used as study models, and a specimen was destructively sampled every 24 hours, starting immediately after death. Both the necrophagous insect specimens (adults and larvae) and the tissue from the rat small intestine were sampled and taxonomically and genetically identified. Furthermore, the air and soil temperature, relative humidity, precipitation rate, and soil pH were recorded daily.

Necrophagous insect species were absent in March, given the low temperatures that did not exceed 10°C in soil, and thus were observed and sampled solely in July. Two Diptera (Muscidae, Phoridae) and two Coleoptera (Leiodidae, Staphylinidae) families were identified, encompassing five and two species, respectively. All Diptera developmental stages were observed and sampled from the remains, beginning with the active decomposition stage, while Coleptera were present only in the last stage.

From the small intestine and insect tissues, the total genomic DNA was extracted and the bacteria diversity was investigated by Illumina® MiSeq® analysis and taxonomy was assigned via QIIME™ platform and Greengenes database. A preliminary screening of the bacterial community structure of the small intestine during decomposition stages, determined by 16S recombinant DNA (rDNA) Denaturant Gradient Gel Electrophoresis (DGGE), led to the identification of two bacterial taxa belonging to Firmicutes of permanent and sporadic occurrence. The analysis of their relative content during decomposition, quantitated by quantitative Polymerase Chain Reaction (qPCR), led to the identification and proposal of putative bacterial markers for postmortem interval estimation.

The results exhibit correlations between the insect species presence and environmental parameters variation, stages of decomposition and bacterial communities' diversity and dynamics, and modification of soil pH throughout the evolution of the decomposition process. This data represents the first study of bacterial diversity determined by 16S rRNA Illumina® sequencing for buried carcasses in a natural environment, proposing microbial markers for forensic investigations based on quantitative evolution of certain bacterial taxa.

Buried Carcasses, Insects, Bacteria

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