A109 Storage and Handling Procedures for Using Soil Molecular Biology as Trace Evidence

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After this presentation, attendees will understand how soil sample handling and storage can alter soil microbial community fingerprints using capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) and fatty acid methyl ester (FAME) analysis.

This presentation will impact the forensic science community because it presents some fundamental knowledge for using soil molecular biology as trace evidence.

The use of soil as trace evidence has been well documented for many of its chemical and physical properties. However, there is less understanding when it comes to soil biochemical properties. One method to explore soil biochemistry is to extract microbial DNA and fatty acids to make a fingerprint. This method is currently in the development stage. This study looks at the effects that storage and handling of soil has on the microbial fingerprint made by soil microbial DNA and fatty acids.

In this project four soil types from Nebraska that vary in soil texture were tested. Each sampling location contained three plots. From these plots, 20 soil cores were collected from a depth of 0cm to 5cm three different times over a one year period. After each collection the soils were sieved and placed in sealed plastic bags in their storage conditions (-80˚C, -20˚C, 4˚C, air dried, oven dried, and freeze dried). As a control, one soil sample from the collection had the microbial community DNA and fatty acids extracted within 36 hours; this became the "fresh" sample to which the storage samples would be compared. Soil was collected at the same location two weeks after the initial collection to identify if rapid changes in the soil microbial community itself exist; to determine if it is possible to go back to a crime scene and recover a similar microbial profile. Two methods have been chosen for the analysis of the soil microbial DNA and fatty acids. The microbial DNA is analyzed by capillary electrophoresis single-stranded conformation polymorphism (CE-SSCP) to form a nucleic acid fingerprint. In theory, CE-SSCP is well suited for forensic use, but is a newer method and still requires detailed testing. The microbial fatty acids are analyzed as fatty acid methyl esters (FAME) to form a lipid-based fingerprint. FAME is a robust and highly used protocol that will support CE-SSCP data.

Thus far, the results from FAME analysis show soil stored at -80˚C and -20˚C show no significant difference from the fresh sample. The other storage methods (4˚C, air dried, oven dried, and freeze dried) showed significant difference when compared to the fresh sample. Samples taken two weeks after initial sampling have shown significant differences in microbial fingerprint compared to fresh samples. These results determine the best way to store soil samples when using soil microbial biochemical molecules is at either -80˚C or -20˚C, as to not change the microbial fingerprint. It also demonstrates the soil microbial community can change rapidly, possibly making it difficult to develop a robust method when using the soil microbial community for a fingerprint when several weeks have passed between crime and investigation. Results from genetic analysis will be presented.

Single-Stranded Conformation Polymorphism, Fatty Acid Methyl Ester, 16S rDNA

 ** Presenting Author*