G69 Using Ninhydrin to Detect Grave Soil

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Some death investigations commence without knowledge of the location of a body and/or decomposition site. In these cases it is necessary to locate the remains or the site where the body decomposed prior to relocation. Ideally, the location of these sites would be rapid and require little destruction of the scene, such as that achieved with cadaver dogs. However, few options remain if cadaver dogs are unavailable or prove unsuccessful.

Ninhydrin is a compound that is readily available to most investigative agencies, as it can be used to locate latent fingerprints. This use relies on the color change that occurs when ninhydrin reacts with protein-, peptide-, amino-, and ammonium-nitrogen (collectively known as ninhydrin-reactive nitrogen: NRN) left on a surface contacted by skin. Similarly, the decomposition of an organic resource results in the release of NRN into the soil. Considering that a cadaver can comprise as much as 3% nitrogen, there is great potential for NRN to be detected in grave soils. As a consequence, this study hypothesizes that the decomposition of a body would result in a significant increase in NRN in soil.

A field experiment was conducted at two disparate field sites during the dry season (March 2003). Site 1 was comprised of a loamy sand soil (84% sand, 11.1% silt, 4.9% clay) and was located in Yabulu, Queensland, Australia. Site 1 receives an average rainfall of 140 mm during the dry season (March-October) and average maximum/minimum temperature equals 22.9 °C/16.7 °C. Site 2 was comprised of a sandy soil (97.7% sand, 1.3% silt, 1% clay) and was located in Pallarenda, Queensland, Australia. On average, site 2 receives 120 mm of rainfall during the dry season and the average maximum/minimum temperature is 26.9 °C/16.4
°C. Grasses with scattered trees dominated the resulting vegetation at the two sites, as is typical of a tropical savanna ecosystem. Juvenile rat (*Rattus rattus*: ~18 g) cadavers were buried (2.5 cm) in the centre of a 2 m^2 plot. Grave soil was collected at 7, 14, 21, and 28 days following burial.

To measure NRN, 2 g soil (dry weight) was amended with 8 ml KCl (2 M) and shaken (150 rpm) for 30 minutes. Following shaking, the solution was filtered through a filter paper (#42) into a culture tube. To 1 ml of filtrate, 0.5 ml ninhydrin reagent [0.8 g ninhydrin, 0.12 g hydrindantin, 30 ml dimethyl sulfoxide, 10 ml lithium acetate] was added, mixed, and incubated at 100 °C for 25 minutes. The reaction was stopped with 10 ml 50% ethanol-water (v/v) and absorbance was read at 570 nm. The concentration of NRN was calculated against a leucine standard. To make leucine standard, 0.469 g leucine was dissolved into 1 l distilled water. This contained 50 mcg nitrogen ml^{-1}. Separate 100 ml volumetric flasks were amended with 0, 5, 10, 15, 20 and 30 ml leucine solution, 50 ml of 4 M KCl, and water to make up to 100 ml. These standards contained 0, 250, 500, 750, 1000 and 1500 mcg nitrogen.

Cadaver burial resulted in a 4-6 fold increase in the concentration of NRN in grave soil. This increase was observed within seven days of burial and remained constant until the end of the experiment (day 28), by which time the cadaver had been skeletonized for a minimum of 14 days. This rapid and stable increase in NRN has great potential to become a standard investigative tool, considering that the analysis of NRN in grave soil can be conducted in less than one hour.

Forensic Taphonomy, Decomposition, Nitrogen

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