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# The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geoscience



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## ABSTRACT

Forensic geoscience is concerned with the analysis of geological materials in order to compare and exclude environmental samples from a common source, or to identify an unknown provenance in a criminal investigation. Diatom analysis is currently an underused technique within the forensic geoscience approach, which has the potential to provide an independent ecological assessment of trace evidence. This study presents empirical data to provide a preliminary evidence base in order to be able to understand the nature of diatom transfers to items of clothing, and the collection of transferred diatom trace evidence from a range of environments under experimental conditions. Three diatom extraction methods were tested on clothing that had been in contact with soil and water sites: rinsing in water (RW), rinsing in ethanol (RE), and submersion in H<sub>2</sub>O<sub>2</sub> solution (H). Scanning electron microscopy (S.E.M.) analysis was undertaken in order to examine the degree of diatom retention on treated clothing samples. The total diatom yield and species richness data was recorded from each experimental sample in order to compare the efficacy of each method in collecting a representative sample for analysis. Similarity was explored using correspondence analysis. The results highlight the efficiency of H<sub>2</sub>O<sub>2</sub> submersion in consistently extracting high diatom counts with representative species from clothing exposed to both aquatic and terrestrial sites. This is corroborated by S.E.M. analysis. This paper provides an important empirical evidence base for both establishing that diatoms do indeed transfer to clothing under forensic conditions in a range of environments, and in identifying that H<sub>2</sub>O<sub>2</sub> extraction is the most efficient technique for the optimal collection of comparative samples. There is therefore potentially great value in collecting and analysing diatom components of geoforensic samples in order to aid in forensic investigation.

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## 1. Introduction

The analysis of trace evidence in forensic geoscience can contribute valuable information to a criminal investigation, especially in the search to exclude a suspect from a crime scene or in the attempt to profile an unknown forensic environment [1]. The examination of microscopic components of soils and sediments can provide useful circumstantial evidence in a range of experimental and case work scenarios [2–5]. Relatively established techniques, including quartz grain surface texture analysis [6–9], palynology [10–12], and soil geochemistry [13,14]; are increasingly

complemented by new methods in the examination of both the organic and inorganic components of a trace geoforensic sample. More recent research is now considering the importance of new geoforensic methods including microbial DNA profiling [15,16], mycology [17], and alkane signatures [18,19]. In comparison, however, diatom analysis has been relatively limited in its forensic application to date.

The value of diatom evidence to geoforensic enquiry lies in the multiple environmental characteristics that can be inferred. Diatoms are unicellular microscopic algal organisms which are widely distributed and naturally abundant in a range of aquatic and terrestrial environments [20]. Individual diatom species and population assemblages are diverse and environmentally specific due to their sensitivity to multiple variables including light, nutrient availability, pH, and salinity [21]. The hardened silica cell wall (SiO<sub>2</sub>) is resistant to decay and retains diagnostic features enabling species identification and forensic comparison [22]. Furthermore, the

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microscopic nature of diatoms increases their potential for use in a forensic capacity. It is highly unlikely that the transfer of diatom traces from a crime scene will be recognised by a perpetrator, enhancing the potential for diatoms to be recovered as evidence.

Diatoms have been established as reliable and naturally abundant environmental indicators in a broad range of applications including palaeoecological reconstruction [23–25], water quality management [26,27], and climate change research [28]. The main application of diatoms in forensic science is currently pathological, assisting in the diagnosis of drowning as a cause of death [29–33]. Further research has been directed towards the use of algae, and particularly diatoms, in the estimation of the post mortem submersion interval (PMSI) of an item or cadaver recovered from water [34,35]. Fossil diatoms have also been recognised as important tracers in soils [36] and anthropogenic materials including paints, pesticides, and safe ballasts [37].

While diatom analysis has been used in various case work examples [38,39], little experimental research is currently observed within the forensic geoscience literature [40]. This echoes the forensic palynology literature which included very little experimental research until 2005 [3,41–43] in contrast to extensive case work examples [11,12,44,45]. Forensic palynology is now a well established field of enquiry with such experimental studies crucial in providing a sound evidence base for the collection and interpretation of pollen evidence in order to provide valuable intelligence. A similar research focus in forensic diatom analysis is essential to provide reliable data towards developing this independent technique for the ecological assessment of geological materials in criminal investigation. Experimental consideration of diatoms in pertinent contexts such as the transfer of diatoms from various source habitats to recipient surfaces, and the recoverability of particulates from those items for forensic analysis contributes to the appropriate interpretation and presentation of evidence in a court of law [46,47].

This paper aims to examine the transfer of diatoms to clothing in a range of aquatic and terrestrial environments, and determine an effective technique for the collection of diatom evidence for forensic comparison. Clothing was determined an appropriate recipient surface to examine diatom transfer due to its frequent presence at a range of crime scenes [48]. The optimal recovery and analysis of trace evidence from clothing is therefore imperative for the reliable comparison and exclusion of samples in forensic geoscience.

In this study, three methods were tested on cotton t-shirts in contact with multiple water and soil sites. The traditional method of rinsing with water (RW) [37] was compared to rinsing with ethanol (RE) (suggested as most efficient in previous research by Uitdehaag [40,49]), and hydrogen peroxide extraction ( $H_2O_2$ ) (adapted from ecological diatom investigation [50,51]). The efficacy of each extraction method was assessed through consideration of the total diatom valve count yielded in each experimental sample, the species richness of each experimental sample when compared to a control, and the similarity of sample composition as determined by correspondence analysis. The impact of submersion time upon the transfer of diatom evidence was also examined. In aquatic contexts, t-shirt samples were submerged for 3 min, 30 min, 3 h, and 24 h; in order to replicate different forensic transfer scenarios. Residual clothing samples were later examined under scanning electron microscopy (S.E.M.) to assess the number of diatoms still adhered to clothing following each treatment.

## 2. Materials and methods

### 2.1. Sample collection

Samples were taken from three aquatic and four terrestrial sites. 500 ml of water and aquatic vegetation was collected from two water

bodies: a small garden pond (P) and a small stream (S) (Hertfordshire, UK). A cleaned diatom concentrate was also used, obtained from an oligotrophic Greenland lake (GL). Both UK sites were relatively secluded and considered relevant to forensic investigation based upon casework examples within the literature [38,39].

Sections of new (unused) 100% cotton t-shirt were immersed in 300 ml of water from each site for 3 min, 30 min, 3 h, and 24 h. T-shirt samples were then removed, dried for 48 h and stored. The different times were chosen in order to replicate multiple forensic scenarios including brief contact when a perpetrator leaves a crime scene (3 min), and an extended time period (24 h) reflecting the disposal of a body or evidence in water [52].

Two locations at both Ravenscourt Park (RCP) and Clapham Common (CC) were sampled for soil diatoms. Sections of t-shirt were pressed against the soil surface for sixty seconds in order to replicate a forensic reality in which diatoms may transfer to clothing following an assault or struggle on the ground. The top 2 cm of soil was collected to provide a comparative control sample.

### 2.2. Control sample preparation

Control samples were prepared following Renberg [50]. 1 g of control site sediment or 20 ml of water from control sites were treated in a water bath with 20 ml  $H_2O_2$  (30%) for 3 h. The samples were then washed with deionised water.

### 2.3. Experimental sample preparation

Three treatments were performed on individual 1 cm<sup>2</sup> sections of each t-shirt.

T-shirt samples were added to plastic flasks containing either 150 ml deionised water (RW) or 70% ethanol (RE). The flasks were shaken for 24 h at 100 rpm (LuckhamR100/TW) before the t-shirt was removed, dried, and stored. The solution was then centrifuged (1200 rpm), washed with deionised water, and the remaining supernatant discarded. The solution was prepared for diatom analysis as detailed below using known dilutions.

A third t-shirt sample for each location was treated by  $H_2O_2$  digestion in a water bath. 20 ml of  $H_2O_2$  (30%) was added to a test tube containing the t-shirt sample and heated in a water bath for 3 h at 70 °C. The t-shirt was then removed and stored in distilled water. The solution was processed as above.

At all stages of sample collection and preparation for analysis, appropriate measures were taken in order to ensure contamination between samples was avoided, in line with standard forensic procedures [53].

### 2.4. Analysis

A 0.5 ml subsample was prepared on a cover slip, mounted with Naphrax™, and analyzed under binocular microscope at 1000× magnification. All individual diatom valves observed in the subsample were identified [54–57] and counted. The estimated diatom concentration in a 5 ml sample was then calculated in order to appropriately compare the assemblage, the estimated total individual diatom count (expressed per cm<sup>2</sup> of clothing), and the species richness of experimental samples with control sites.

The efficiency of each technique and the impact of submersion interval upon transfer were then compared by calculating the percentage of all diatoms extracted. The similarity in species composition across both experimental and control samples from each site were compared using correspondence analyses [58].

### 2.5. S.E.M. examination

Scanning electron microscopy (S.E.M.) was used to examine the persistence of diatom particulates on clothing that had been

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