The Value of an Empirical Approach for the Assessment of Diatoms as Environmental Trace Evidence in Forensic Limnology

Kirstie R. Scott,* [1, 2, 3] Ruth M. Morgan,[1, 2] Vivienne J. Jones,[3] Aoife Dudley,[4] Nigel Cameron,[3] and Peter A. Bull[4]

[1.] Department of Security and Crime Science, University College London
[2.] Centre for the Forensic Sciences, University College London
[3.] Environmental Change Research Centre, University College London
[4.] Oxford University Centre for the Environment, University of Oxford

* Corresponding author k.scott.12@ucl.ac.uk

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Abstract

Environmental trace evidence is often encountered during a forensic investigation and is acknowledged to have the potential to contribute valuable circumstantial information pertaining to the context of an individual criminal event. Although traditional study has focused upon the analysis of terrestrial soil and sediment traces, there is growing potential for the forensic assessment of aquatic crime scenes, particularly those within freshwater environments. This paper outlines the current applications of limnology, particularly algae and diatom analysis, within forensic science and introduces new and ongoing research within the field. Two empirical studies are presented which highlight the importance of developing evidence bases within freshwater trace evidence analysis. These studies demonstrate the analytical capability of the Scanning Electron Microscope (SEM) at various stages of an investigation: in the initial screening and collection of an evidential sample from clothing (1); and in the analysis of preserved diatoms following various levels of their exposure to fire damage (2). The results highlight that the SEM provides a valuable tool during the initial stages of an investigation, determining the presence and abundance of a range of environmental indicators and directing further strategy for the more in-depth collection and analysis of a forensic sample. Furthermore, the preservation of diatoms adhering to clothing following prolonged exposure to fire, indicates that efforts to collect any destroyed evidence are worthwhile given the potential to recover freshwater traces over extended time scales. Finally, the value of adopting an empirical approach for the development of a forensically relevant evidence base within forensic limnology, and the importance of having an appreciation of the legal implications for the interpretation and admissibility of freshwater evidence is presented.

Introduction

The scientific examination of environmental particulates transferred from a pertinent forensic location to a person or item of interest, contributes important circumstantial evidence in legal investigation. The concept that "every contact leaves a trace" is one of the guiding principles of forensic science, and it is acknowledged that there have been significant advances in our capacity to not only identify an initial transfer of evidence, but also in the development of increasingly sensitive techniques for evidence collection and analysis (Stoney and Stoney 2015). The assessment of environmental materials within forensic geoscience, has primarily focused upon soils, sediments, and pollen in terrestrial contexts (Ruffell 2010; Morgan and Bull 2007a). The presence and abundance of biological and physical traces in aquatic environments, although valuable, are arguably less studied as trace evidence in the published literature. The spatio-temporal variability of algal communities in freshwater environments provides valuable ecological intelligence across a diverse range of forensic disciplines. Microscopic algal organisms, including diatoms, have been used in forensic pathology to diagnose a cause of death by drowning (Pollanen 1997, 1998; Krstic et al. 2002; Cameron 2004; Delabarde et al. 2013) and to reconstruct or estimate the post mortem submersion interval (PMSI) of a cadaver recovered from water (Cassamatta and Verb, 2000; Zimmerman and Wallace, 2008). They have also been used in forensic archaeology (Carlie et al. 2014) and environmental forensic disputes related to water quality and algal toxin poisonings (Codd, 2000; Gessner et al. 1997; Graham et al. 2002). The assessment of diatoms and other algal groups as a form of trace evidence for crime reconstruction is a relatively novel development, and one which offers great value to the field of forensic geoscience and the forensic sciences more broadly.

Several case examples within the literature consider the successful application of freshwater algae as trace evidence in various investigative scenarios (Siver et al. 1994; Cameron 2004), however empirical research studies generating additional intelligence within the field are relatively recent (Scott et al. 2014). Growing calls within the forensic science community, from both academics and policy makers, increasingly identify the need for research to establish empirical evidence bases for forensic application (Mnookin et al. 2011; Annual Report of the Government Chief Scientific Adviser 2015). The knowledge bases within "parent disciplines" such as botany, geography, biology, and chemistry are well established, however similar approaches are required for application within forensic contexts. This contributes towards the development of guidelines for the efficient collection and accurate analysis of different forms of trace evidence, enabling transparent and robust interpretation of evidential significance within the context of an individual case. This works to increase the validity and reliability of evidence presented as intelligence to investigators, or as probabilistic evidence in court (Morgan et al. 2009).

This paper outlines a collaborative approach towards the development of an empirical evidence base to support and enhance the current use of diatoms and freshwater algae as indicators in forensic limnology. The role of freshwater trace evidence within environmental forensic science is presented, and the value of experimental research studies is illustrated with reference to two examples which consider the use of electron microscopy for the collection and analysis of diatom traces as evidence.

Environmental trace evidence

The legal study of environmental evidence has predominantly been defined as "forensic geoscience"; the utilization of theories and techniques developed within the geosciences (geography, geology, botany, ecology) and applied to criminal and civil judicial proceedings

(Morgan and Bull 2007a). Although forensic geoscience also considers the search and investigation of large spatial areas (Pringle et al. 2012), the "micro" scale approach to trace evidence collection and analysis is the focus of this paper.

The scope of forensic geoscience

Forensic geoscience has developed to appropriately consider the biological and chemical components of the earth's surface alongside the traditional physical analysis of rocks, sediments, and soils (Ruffell 2010). As such the study of various ecological and environmental traces including pollen, microbes, and soil minerals are encountered within the remit of forensic geoscience. Soil is the most frequently recovered environmental trace evidence due to its abundance in domestic and public locations and its tenacity to transfer and persist on a range of evidential items. The forensic study of soils and sediments encompasses the analysis of the physical, chemical, biological, and anthropogenic components of a sample using various techniques (Cox et al. 2000; Ruffell and McKinley 2005; Morgan et al. 2006, 2007, 2008; Hawksworth and Wiltshire 2011). The individual properties of a soil sample have traditionally been observed using light and electron microscopy methods (Dawson and Hillier 2010). Optical analyses allow for the examination and identification of individual particulates such as quartz grain surface textures (Bull and Morgan 2006), pollen grains (Wiltshire 2015), and diatom valves (Scott et al. 2014). A growing area of research is directed towards improving the range and efficiency of those techniques available for the analysis of geoforensic evidence types. Recent studies have focused upon the development of automated scanning techniques for the rapid identification and quantification of the minerals or quartz grains in a soil sample (Pirrie et al. 2009; Newell et al. 2012); as well as the use of biogeochemical and genetic techniques, testing the extracted eDNA or rRNA from a homogenized soil or microbial profile (Young et al. 2015, 2016).

The philosophical approach to environmental trace evidence

The dynamic nature of an environment leads to the incorporation of natural and anthropogenic materials from various provenances; characterizing a site to such an extent that it becomes highly distinctive and useful for forensic comparison. This spatio-temporal variability creates discrete and ordered "micro-terrains," affording great value for forensic science (Morgan and Bull 2014). Given the variability of environments (even those with similar underlying geology and land use), analysis of the microscopic characteristics of a trace soil or water sample can be used effectively in a range of forensic investigations. Examples within the literature include homicide (Smith et al. 2002), serious assault (Siver et al. 1994), poaching and wildlife crime (Morgan et al. 2006), burglary (Mildenhall 2006), and the reconstruction of international war crimes (Brown 2006).

Whilst natural variability affords great value within environmental forensic science, the analysis of trace evidence and the interpretation of results should be approached with caution. Any environment cannot be considered to be "unique," hence forensic investigation should pursue one of two goals: the exclusionary comparison of evidential samples from a previously identified location [1]; or the exclusionary determination of provenance where a source location is unknown [2] (Morgan and Bull 2007a). Furthermore, the analysis of earth surface materials during forensic enquiry should operate within the tenets of the philosophical framework outlined by Morgan and Bull (2007b). Notably, in any comparative analysis, exclusionary approaches should be taken and a range of analytical techniques independently employed in order to examine the ubiquitous and the rare components of a trace sample.

Forensic ecology

The dynamic complexity of parent soil material provides distinct microhabitats where communities of organisms live and reproduce. The study of these organisms within the forensic geoscience framework is termed forensic ecology; with indicators including pollen, fungi, algae and bacterial profiles influenced by the chemical and physical characteristics at a given site (Wiltshire 2009). Fluctuations in the presence, abundance, and diversity of microorganisms is directly related to abiotic conditions such as soil chemistry, light and moisture availability; as well as biotic factors including other plants, animals, and human interventions.

The forensic study of ecological indicators is a key component of environmental trace evidence analysis. The abundance of vegetation and plant traces throughout terrestrial and aquatic environments provides substantial opportunity for transfer to evidential surfaces. When recognized and appropriately recovered as evidence, botanical material offers a valuable biological technique for the comparison and exclusion of samples and in the profiling of an unknown environment (Wiltshire, 2009). This has primarily been observed through pollen analysis which has been widely used in casework across the U.S. and Europe (Smith et al. 2002; Brown, 2006; Wiltshire 2006). Concurrent empirical research considers the transfer and persistence dynamics of pollen evidence in various forensic scenarios (Riding and Rawlins, 2007; Morgan et al. 2014a, 2014b), and generates knowledge of the diverse range of circumstances in which pollen may be recovered during an investigation. This contributes towards the development of an empirical evidence base for the robust and transparent interpretation of palynology in court.

Aquatic ecology

Aquatic environments are notoriously challenging when encountered during a forensic investigation, due to their complex physical and chemical dynamics. The presence of ecological communities in the form of plants or animals varies substantially in line with water depth, turbulence, nutrient availability and the presence of predators. Two microhabitats are primarily identified within a water body: planktonic (suspended) and non-planktonic (often bottom dwelling, attached or motile within the surface sediment) communities. Aquatic organisms including ostracods, barnacles, bacteria, amphipods, and aquatic insects have been encountered in forensic research and casework pertaining to decomposition, cause and time since death, and establishing provenance (Merritt and Wallace 2000; Magni et al. 2015; Anderson and Bell 2016).

While pollen may still be identified in a water sample, the forensic assessment of algae is of greater botanical value in those crime scene environments involving marine or freshwater (Hardy and Wallace 2012). The environmental ubiquity of algal communities, including diatoms, emphasizes their scope as trace evidence indicators. Although over 70% of the earth’s surface is covered in water (USGS 2016), examples of geoforensic investigation in aquatic environments are relatively few. Case studies relating to the analysis of aquatic trace evidence are limited, with research arguably overlooked due to the complexity and often inaccessibility of seas, oceans, and freshwater ecosystems.

The trace evidence framework

The need for an empirical evidence base to support the robust use of environmental particulates can be observed throughout the entire forensic process: from the collection of transferred evidence to its successful presentation in court (Inman and Rudin, 2002). Experimental research underpins the scientific understanding of the dynamics of trace evidence. These studies provide an evidence base to appreciate how evidence originates, increases the validity and reliability of collection and analytical techniques used, and allow for the appropriate interpretation of evidence within the context of individual crime reconstructions (Mnookin et al. 2011). Such work establishes transparent and defensible scientific foundations, contributing to the legal admissibility of evidence in court in line with the Daubert criteria, utilized in a number of U.S. states and advocated in the 2011 Law Commission Report of the UK (Black et al. 1993; National Academy of Sciences Report 2009).

To ensure the appropriate consideration of diatoms and freshwater algae as trace evidence indicators and subsequently define standards for the interpretation of evidence, it is important to dedicate resources towards experimental research (Morgan et al. 2009). Maintaining a close synergy with current forensic practice will contribute towards the understanding of the specific circumstances accompanying a criminal event in an aquatic environment whilst acknowledging the wider context within which forensic science operates. The study of algae during forensic investigation Algae and other aquatic plants are ubiquitous and abundant microorganisms in almost all water environments. Since vast areas of the earth's surface are covered in marine and freshwater, contained in the oceans, seas, lakes, rivers, and ponds; algae account for more than half of total primary production worldwide (Hoek et al. 1995). Ecologists have long recognized the critical importance of algae in the functioning of aquatic ecosystems, supporting the growth and diversity of virtually all other organisms. This ecological basis has led to their study as trace evidence indicators in forensic science and crime reconstruction.

Algal diversity

Algae are presented in various sizes, colours, and morphologies, which are adapted to distinct ecological tolerances and habitat characteristics. Recognizing algal presence in an environment, categorising and identifying organisms to group, genus and species-level, and realising the influence of ecological distribution patterns, often requires specialist knowledge. Such knowledge should be sought and valued when algal communities are encountered in forensic investigation (Hardy and Wallace 2012).

Most algae are unicellular and microscopic and thus invisible to the naked eye and the potential perpetrator of a crime in an aquatic environment (Round 1970). Multiple cells can also be observed in filamentous arrangements, often floating on the surface of ponds or lakes or attached to submerged strata; whilst larger macro algae are viewed as individual plants. In the investigative approach of an aquatic crime scene, the presence of algae should always be presumed and attempts made for the collection of evidence (Cox 2012). Larger plants and filamentous algae stains adhered to an evidential surface may be immediately apparent, however microscopic unicellular organisms may also be embedded and otherwise go unnoticed.

Algae are pigmented, with common species appearing and initially identified based upon a green, red, brown, golden-brown, or blue-green colour. Green algae are the most abundant algal group with an estimated 7500 species worldwide. Groups and species may be unicellular, multicellular, and colonial; with common taxa including Closterium spp., Pediastrum spp., Volvox spp., Desmidiales spp., and Chlorella spp. (Hoek et al. 1995). Green algae are ubiquitous in a range of environs including marine and freshwater, snow, and terrestrial locations. Comparatively, select algal groups are more abundant in oceanic environments and may therefore be less frequently encountered in forensic limnology. For example, red and brown filamentous algae are commonly presented as marine seaweeds, while 90% of unicellular dinoflagellates exist as marine plankton. Species including Ceratium spp. and Peridinium spp. can, however, frequently appear in samples of freshwater plankton.

Blue green algae, or cyanobacteria, are diverse in their form and distribution worldwide. Some species generate powerful algal toxins and blooms which can prove fatal to wildlife populations and dangerous when consumed or exposed to humans (Graham et al. 2010). Growth in the prevalence of these species is fuelled by increases in nutrient runoff (fertilizers etc.), sunlight availability, and warm temperatures (Codd 2000). Although beyond the remit of forensic limnology, harmful algal “red tides” of toxin-producing dinoflagellates may bloom in oceanic and coastal environments. The subsequent accumulation of dinotoxins in fish and shellfish can lead to PSP (paralytic shellfish poisoning) and related conditions when consumed by humans (Gessner et al. 1997). Blue-green algae may therefore be encountered in criminal or civil forensic investigation: identifying a cause of death or serious illness, or in determining the liability for affected water bodies on private property (Hardy and Wallace 2012).

Environmental distribution

Algae are found in almost all aquatic environments. Due to their diversity, algal species can tolerate a wide range of ecological conditions and are observed in extreme hot and cold environments, in terrestrial and aerial environments, and as endosymbionts within other plants and animals (Graham and Wilcox 2000). In freshwater systems, algae and diatoms flourish at different stages of the water column. Those species found suspended within the water are termed planktonic, whilst non-planktonic species are often attached to or growing on the surface of submerged sediments (Round et al. 1990). Microhabitats including rock surfaces, submerged vegetation, and sandy sediments also support discrete algal communities. The distribution of freshwater algae can be further influenced by turbulence within the water. For example, certain species may develop physiological adaptations enabling or resisting transportation in flowing river environments (Jones 2007). Variation in the distribution of algae between and within an ecosystem has important implications for forensic investigation. The presence of primarily benthic species on the clothing of a criminal perpetrator might infer an initial contact with the surface bed of a river, lake or pond. Such information is not only valuable in the direct comparison and exclusion of crime scene samples, but also provides circumstantial evidence for reconstructing the circumstances surrounding a criminal event.

Diatom assessment

Diatoms are a group of unicellular eukaryotic golden-brown algal organisms, characterized by their distinctive silica cell wall (Figure 1). They are the most species-rich group of algae with an estimated 12,000 species worldwide (Jones 2007); widely distributed in both aqueous and terrestrial environments (soils, exposed rock, tree bark etc.) and in natural and domestic settings. The hardened cell wall ensures that diatom valves are extremely resistant, with “fossil” diatoms preserved in the sediment record and extensively used to reconstruct past environmental change (Battarbee et al. 2001; Birks et al. 2004; Juggins and Birks 2012).

The size of diatoms ranges from approximately 5–500μm, with the morphology and ornamentation of the silica cell wall utilized in diatom classification and identification (Battarbee et al. 2001). Each diatom cell is contained within two separate valves, joined together in a box like structure. Using light and electron microscopy, features of the diatom valve including the shape (centric or pennate), raphe (central spine), and arrangement of pores and striae; can be observed and used to record the genus or species of an individual valve (Figure 1) (Jones 2007). The diversity of a diatom assemblage and the complex arrangement of any identifying features makes accurate species identification very difficult, with frequent debate amongst scientific experts. Taxonomists are continuously developing identification guides and databases to ensure the appropriate documentation of new and existing species. Such resources should always be consulted by a forensic examiner when diatoms are recovered in a criminal investigation.

The individual diatom species and overall population assemblage at a site are diverse and, like all algal groups, are controlled by ecological response to various physical and chemical factors, including silica availability (Reynolds 2006). The tendency to find particular diatoms under relatively specific environmental conditions lends great value to the forensic profiling of an unknown location.

Figure 1. SEM micrographs highlighting the diversity and ornamentation of the silica cell wall. Freshwater diatom taxa are identified as: A) Pennate species Navicula lanceolata (x2,200 magnification); B) Centric species Stephanodiscus spp. (x8,500 mag); C) Pennate Epithemia adnata (x2,500 mag); D) Pennate Cocconeis placentula (x2,000 magnification); E) Pennate Amphora pediculus (x9,000 mag); F) Centric Cyclostephanos spp. (x5,000mag). All images are authors own.

Value for forensic science

The importance of collecting and analyzing algal evidence in forensic science can be attributed to three main traits:

- the wide distribution and natural abundance of organisms in a range of environments;
- the diverse nature of individual taxa and the overall species composition at a site in line discrete ecological conditions;
- and the microscopic size of individual organisms—enhancing the potential for transfer and limiting the possible removal of evidence (Scott et al. 2014).

Algal indicators including diatom valves, chrysophyte scales and stomatocysts, and dinoflagellate thecae, offer additional forensic value based upon their resistance to desiccation and the retention of distinctive features for identification. Due to this natural durability, diatoms may offer forensic intelligence even when the collection of evidence is delayed during the nature of a criminal investigation.

The forensic study of algae offers valuable ecological information during a range of investigations. Traditionally, planktonic organisms (particularly diatoms and green algae) have been used to support the determination of a cause of death by drowning within forensic pathology (Incze 1942; Peabody 1980; Pollanen 1997, 1998; Krstic et al. 2002; Delabarde et al. 2013). Empirical studies have complemented current practice through the development of new and more sensitive techniques, and by attempting to understand and overcome the limitations associated with the diatom test (Hurliman 2000; Yen and Jayaprakash 2007; Kakizaki and Yukawa 2015). A similar empirical focus exists within forensic anthropology, where research has aimed to utilize the temporal variation of algal groups, for the estimation of post mortem submersion interval in aquatic environments (Cassamatta and Verb 2000; Haefner et al. 2004; Zimmerman and Wallace 2008).

Although algae cannot act to individualize or identify a perpetrator, when appropriately collected and analyzed, they can provide useful contextual information in aid of crime reconstruction (Peabody and Cameron 2010). The deposition of algal organisms in the internal organs or their adherence to an evidential surface such as clothing or footwear, can ascertain information on when, how, and where a crime has taken place (Yoshimura et al. 1995; Cassamatta and Verb 2000; Cameron 2004).

Although several algal groups have been encountered to some extent within forensic limnology, diatoms are the most extensively studied within pathology, anthropology, and trace evidence analysis. This paper will continue to discuss the application of diatoms as trace evidence indicators in forensic casework, and introduce the growing body of empirical research directed towards understanding their transfer and persistence dynamics, and developments for the optimal collection and analysis of an evidential sample.

Diatoms as trace evidence indicators

In the environments where diatoms are naturally occurring, the transfer and persistence of particulates to an evidential surface such as clothing or footwear, can contribute valuable exclusionary evidence to discriminate amongst a suspect or victim and a crime scene (Peabody and Cameron 2010). The mining of fossil diatom deposits, for use in anthropogenic products such as paints, pesticides, filters, and construction materials, presents additional possibilities for diatom transfer and presence in forensic samples.

Transfer and persistence

Despite the presence of diatoms in aerial and terrestrial soil environments (Johansen 2011), all published accounts of their use as trace evidence indicators in forensic science are focused upon marine or freshwater scenes. Where materials have been submerged or there is contact with littoral or riparian sediment or vegetation, any potential diatom transfer can be used to profile and identify the type of habitat involved (Cameron 2004). When compared to a known crime scene environment, the analysis of freshwater diatom traces recovered from the clothing or footwear of a suspected perpetrator should consider both the individual species present and the composition (% abundance) of the overall species assemblage. This approach can infer knowledge pertaining to the type and extent of the initial transfer of material and deduce the likely activity of a perpetrator during and in the aftermath of a crime. Such information may prove valuable during crime reconstruction in freshwater environments. Published geoforensic case studies in forensic limnology are relatively rare. When collected and analyzed as trace evidence, freshwater diatoms have provided circumstantial information contributing towards forensic reconstructions of murder, serious assault, police brutality, alibi verification and serial burglary events (Siver et al. 1994; Cameron 2004; Stam 2009; Peabody and Cameron 2010). The presence of diatoms in a number of anthropogenic materials further contributes to their abundance in the environment, and provides additional opportunity for their study in forensic science and crime reconstruction. For example, the past use of diatomite in the insulation of safes, led to a number of burglary cases where diatom evidence contaminated the clothing of a perpetrator and provided an association with the crime scene (Peabody 1971, 1977).

Empirical research addressing the dynamics of trace evidence in general, and diatoms more specifically, particularly their transfer, persistence, and preservation in a range of forensic scenarios, is essential to ensure the optimal collection, analysis, and interpretation of evidence later in an investigation (Uitdehaag et al. 2010; Scott et al. 2014). This foundation has started to be established in geoforensic trace evidence research, including quartz grain surface texture analysis and forensic palynology (Bull and Morgan 2006; Morgan et al. 2008, 2014a, 2014b). These approaches offer an additional basis upon which to aid the interpretation of environmental trace evidence, enhancing the value of intelligence offered to investigators, and the overall assessment of evidential significance when applied to the individual circumstances of a case.

Collection and analysis protocols

A number of techniques for the collection of diatom valves adhered to clothing have been outlined in the literature (Uitdehaag et al. 2010; Scott et al. 2014). Research has focused upon the use of several mechanical and chemical methods, including rinsing with water and ethanol, and the digestion of fabrics using hydrogen peroxide (H2O2), sulfuric acid (H2SO4), and nitric acid (HNO3), for the extraction of diatoms from cotton garments. A recent study considered the collection of diatoms transferred to clothing following exposure to both freshwater and soil environments (Scott et al. 2014).

The treatment of clothing using hydrogen peroxide (H2O2) was found to be the most successful and consistent in the extraction of a diatom assemblage with high levels of similarity to the comparative control samples tested (Figure 2). Chemical digestion with H2O2 has traditionally been used in the recovery of diatoms from human tissues in drowning cases (Auer 1988; Ming et al. 2006), as well as in the preparation of samples for palaeoenvironmental reconstruction (Renberg 1990). The scientific examination of transferred trace evidence following H2O2 treatment, establishes the reproducibility of the technique for application in several forensic approaches aimed towards diatom recovery. This enables an empirical, evidence based justification for using this collection method, contributing to the admissibility of recovered diatom evidence in court. Wherever possible, a representative control water sample should be collected during the initial investigation and processing of a crime scene, to enable later comparison to any evidential samples recovered.

While recent research has improved the capability to recover a representative diatom sample from clothing as an evidential surface, comparable research must also consider evidence collection from alternative surfaces, such as footwear. The sole of a shoe provides a direct contact with any benthic diatom communities present when a perpetrator or victim is standing in water. To our knowledge, no published studies currently exist concerning the collection of diatom traces from footwear, with great potential to contribute meaningful scientific results for the legal interpretation of evidence.

Figure 2. The hydrogen peroxide recovery technique for the collection of diatoms from clothing as outlined by Scott et al. (2014), where H2O2 refers to hydrogen peroxide and HCl is hydrochloric acid. The preparation of blank samples is recommended throughout the process, in order to exclude the potential for any contamination.

1. Dry several samples of a clothing garment overnight, in covered petri dishes in a fume hood
2. When dry, identify and collect small (1cm2) subsamples of clothing material for further treatment (visible staining may be observed)
3. Add each subsample to an individual disposable centrifuge tube, add 20ml of H2O2 (30%), and heat to 70°C in a water bath for four hours
4. Remove the material, rinse with distilled water, and store each subsample in case further analysis is required
5. Add a few drops of HCl (10%) to the sample solution and top up with distilled water
6. Centrifuge for 4 minutes at 1200rpm, aspirate the remaining supernatant and re-suspend any particulates with distilled water. Repeat four times.
7. Using a calibrated micropipette, transfer a known quantity of solution onto a glass coverslip and leave to settle overnight
8. Mount the coverslips onto glass slides using a high refractive index mountant (>1.7) and examine with phase contrast or bright field microscopy.

The analysis of diatoms in environmental and forensic science has primarily been by light and phase contrast microscopy (Battarbee et al. 2001). The H2O2 method adopted in the preparation of samples removes any organic material allowing for clear observation of the diatom cell and any persisting silicates such as phytoliths and chrysophyte cysts and scales. The quantitative examination of a sample is imperative when conducting a “compare and exclude” investigation based upon the abundance and composition of the diatom species present. To quantify a count for comparison, the concentration of the solution and the number of transects counted should always be noted in order to standardize counts and ensure the accurate representation and communication of data (Scott et al. 2014). Qualitative analysis of species presence/ abundance may also prove useful when drawing environmental inferences based upon the diatoms noted, or in those cases where few valves are recovered at all.

Electron microscopy provides a high resolution approach for diatom identification, and is often used in the examination of environmental forensic indicators (Morgan et al. 2006; Bull et al. 2006). The quantitative analysis of diatom traces has not been attempted using the Scanning Electron Microscope (SEM) to date, although there is great potential to develop an efficient method for the simultaneous examination of evidence following the recovery of various algal indicators.

Two examples are presented to illustrate the scope and value of an electron microscopy approach for the screening and initial collection of freshwater trace evidence from clothing (1) and the analysis of any diatom traces preserved on garments following their exposure to fire (2). Scientific enquiry into the potential use of the SEM as a quantifiable analytical tool at various stages of the investigative process, will extend the range of techniques available during crime reconstruction. In turn, this will contribute towards the creation of an evidence based and transparent foundation to guide the collection of evidence, based upon the individual circumstances of a forensic event, and its interpretation in court.

Experimental study one: Evidence collection

The purpose of this initial study was to investigate the potential for the efficient collection and analysis of diatoms via SEM preparation procedures and high magnification observation. The recovery of freshwater trace evidence from evidential surfaces has primarily focused upon oxidizing chemical preparation, removing any organic material for clear observation of the silica diatom frustule under light microscopy (Uitdehaag et al. 2010; Scott et al. 2014). Although effective for the identification of diatom species, the technique is destructive for alternative biological indicators including pollen and most other algal groups. Observing several forms of environmental trace evidence simultaneously can offer multiple lines of enquiry, which may contribute independent and corroborative knowledge during a forensic investigation. As forensic scientists are often dealing with microscopic quantities of evidence, it is important to ensure that the amount and the quality of this evidence is not compromised (Morgan and Bull 2014). Directing resources towards the immediate treatment of samples for diatom isolation, may result in the loss of alternative biological indicators which might otherwise have added exclusionary value for crime reconstruction. This study therefore aimed to examine the use of Scanning Electron Microscopy (SEM) for the initial screening of any freshwater trace evidence transferred to clothing. This initial assessment has the potential to provide valuable early intelligence, identifying priority areas and strategy for the collection of evidence, and guiding subsequent stages of an investigation.

Methods

Two regularly worn cotton and nylon clothing garments were submerged in a brackish retention pond in Elgin, Illinois (USA) for a period of 5 minutes in June 2015. The items were removed, individually packaged, and dried in a controlled environment. Replicate samples were then prepared for microscopic comparison. The first stage of analysis addressed the removal of transferred particulates from clothing for direct visual observation (12 "extract" samples). A 12mm SEM stub (with attached selfadhesive carbon disc) was contacted against the surface of the cotton/nylon clothing a total of 15 times (Figure 3), gold coated, and examined at 1,200x magnification. The in situ examination of particulates adhered or embedded in the weave of the garment provided additional assessment (6 "embedded" samples). In each instance, a 12mm2 subsample of each item of clothing was attached to an SEM stub and prepared for investigation. A total of 18 samples were analysed. For quantification, a known area of each SEM stub was examined and the diatoms counted. These counts were multiplied accordingly to provide an indication of the estimated total number of diatoms recovered from each SEM sample.

Figure 3. Method collection for "extract" samples, recovered from the surface of the garment; and "embed" samples, an adhered subsample of the fabric on an SEM stub

Results

Diatoms were the most commonly identified traces in all samples analyzed. When observed, particulates were recorded and categorized according to their shape (pennate or centric) and their quality (whole or fragmented). A substantially higher number of diatoms were observed in both cotton and nylon "extracted samples," where the SEM stub was contacted to the clothing garment in an attempt to remove surficial trace evidence (Figure 4). For example, up to 10,676 diatoms were observed following extraction from the surface of "Cotton 1," compared to less than 1,058 diatoms in the three replicates for in situ analysis of the same garment. Most of the identified diatoms were pennate, with no centric taxa observed in 4 of the 6 embedded samples. Over 5,800 diatoms were noted in all three replicates from 3 of the 4 garments studied in the removal of evidence. Notably fewer diatoms were observed in the second nylon garment with total counts ranging from 2,405 to 962. Interestingly, a higher proportion of centric diatoms were observed, accounting for up to 60% of the assemblage when compared to "Nylon 1" (30%) (Figure 4).

Figure 4. Total number of diatoms identified in all extracted and embedded samples (per 12mm SEM stub).

Most diatom valves observed were whole in their form, allowing for further indepth species identification if required. Of the 12 extract samples, 65–95% of the diatoms observed were whole, with the highest proportion of fragmented diatoms observed in the "Cotton 1" replicate samples (Figure 5).

Figure 5. The distribution of whole and fragmented diatoms in all extracted and embedded samples (per 12mm SEM stub).

Of the 6 embedded samples examined, fragments were only observed in one sample (Cotton B). Although diatoms were the most abundant trace evidence indicator on the clothing samples, a number of other particulates were observed and noted according to their presence (*) and abundance (**) (Figure 6). Mineral grains were detected in all extract samples and were the only other particulate identified in the six embedded samples. A number of biological organisms were identified in the recovered samples, with pollen and spores (including Pinus spp.) present in all but one sample (Cotton 1C). Green algae species including Pediastrum spp. and Scenedesmus spp. (Figure 7) were also observed, as well as plant and insect fragments, silicate chrysophyte scales (Nylon 2A), and anthropogenic glass particulates (Cotton 1C).

Figure 6. The range of alternative freshwater indicators identified across all cotton and nylon extracted samples. Minerals and pollen/spores were present (*) or abundant (**) in almost all samples. Green alga taxa including Scendesmus spp. were also identified. "Other" indicators included: anthropogenic particulates (Cotton 1C); botanical fragments (Cotton, 1A, 1B); insect fragments (Cotton 1B); and a chrysophyte scale (Nylon 2C)

Figure 7. SEM micrographs of trace indicators observed in extracted samples: A) Unidentified spore (x650 magnification) [Sample: Cotton 2B]; B) Green alga cf. Scenedesmus spp. (x4,500 mag) [Cotton 1A]; C) Anthropogenic glass bead (x4,500 mag) [Cotton 1C].

Experimental study two: Evidence analysis

The second study was designed to consider the potential for the assessment of any preserved diatoms following the destruction of recipient clothing surfaces. SEM analysis may provide detailed assessment of any remaining trace indicators when a recipient surface has been compromised, and no visible evidence is apparent for collection and preparation. Several examples within the literature consider the preservation of environmental particulates following various attempts to remove or dispose of evidence, such as the washing or burning of clothing and footwear (Bull et al. 2006; Morgan et al. 2009). The resistance of materials including diatoms and pollen grains, to mechanical and physical stress contributes to their extensive use in environmental reconstruction. This resistance also provides an empirical basis to consider the preservation of ecological trace evidence following exposure to various damaging processes pertinent to a forensic scenario.

Experimental studies have considered the impact of fire on the availability and quality of quartz grain surface textures and pollen grains in forensic investigation (Morgan et al. 2008, 2014a). The preservation of both evidence types has been disputed during individual legal cases, providing a sound rationale for empirical consideration. Since silica (the main component) of the diatom cell is resistant to temperatures of up to 1200°C (Piperno 2006), it is reasonable to presume that diatoms may retain their characteristic features for identification. However, archaeological study of those diatoms present in the clays used in pottery, suggests that diatoms can be destroyed in the firing process when temperatures exceed 800°C (Jansma 1981). The second part of this study therefore aimed to consider the extent of diatom preservation following various stages of fire damage (at temperatures of up to 850°C) to clothing. This empirical focus will offer a valuable initial insight into the temporal dynamics of diatom trace indicators adhered to clothing, and will potentially extend the current applications of the technique in forensic casework.

Methods

A 500ml freshwater sample was collected from the River Beane in Hertfordshire (U.K) in July, 2014. The surface sediment was disturbed to ensure the collection of both planktonic and non-planktonic diatom communities. A number of subsamples were A B C cleaned and prepared using the H2O2 method (Figure 2) to remove any organic material, resulting in 200ml of diatom rich solution.

Individual 1cm^2 swatches of 100% cotton t-shirt were submerged in a beaker with 10ml of the diatom-rich solution for a total of 3 minutes, removed, and allowed to dry overnight in a covered petri dish. Replicate subsamples were then exposed to a flame (up to 850°C) for several stages: 5 seconds contact with fire (Stage 1); approximately 20 seconds contact or until the material was singed (Stage 2); 30 seconds contact or until the material was charred (Stage 3); 1 minute or until the cotton was completely burned to ash. The remaining material (or ash) was attached to an SEM stub with carbon adhesive, gold coated, and examined under the SEM at 850x magnification.

Three control garment samples were also prepared to examine the initial transfer of the diatoms to the cotton, as well as four control water samples to assess the natural distribution of centric and pennate species. A total of 19 samples were examined. To ensure comparable diatom counts, a known area of each SEM stub was counted. The diatoms identified were then multiplied accordingly to provide an indication of the estimated total number of diatoms recovered on each SEM stub.

Results

The diatoms present within all samples were recorded and categorized according to their shape (pennate or centric) and their quality (whole or fragmented). In all four water samples analysed, the proportion of centric diatom taxa was higher than pennate species, accounting for over 60% of the total valves counted (Figure 8).

Figure 8. The distribution, and common centric/pennate diatom taxa, from the four control water samples analyzed.

Although less abundant, the assemblage of pennate diatoms was notably more diverse with common genera including species of Achnanthes, Fragilaria, Navicula, and Nitzschia. By contrast, centric diatoms were primarily Melosira varians and were observed as individual valves or in chains containing several cells. Of the three control clothing samples analysed, an average of 3,124 diatom valves (per cm2 of clothing) was counted (Figure 9).

Figure 9. The total number of diatoms counted following each stage of fire exposure (per cm^2 of clothing).

A decay in the number of diatoms present can be observed with up to 2,002 particulates/cm2 recorded in Stage 1 samples compared to a maximum of 1,522 (Stage 2), 881 (Stage 3), and 641 (Stage 4). This decline is consistent across the three replicates studied. The greatest loss in the number of particulates can be observed between the average control sample and Stage 1 of the experiment. The average persistence of centric and pennate diatoms over the four stages of the study was also considered (Figure 10).

Figure 10. The average persistence of centric and pennate diatom species over the course of the experiment (per cm2 of clothing).

Both groups of diatoms decline over time, although the loss of centric diatom valves is more gradual. For example, following 20 seconds of exposure to fire (Stage 2), 49% of centric diatoms were still observed (of the 100% control sample presence), compared to 33% of pennate species. Centric and pennate species continued to be observed following the complete destruction of clothing at Stage 4 of the experiment, although in lower quantities. For example, 17% (pennate) and 20% (centric) of the sample assemblage was still present across the three replicates. Most diatoms encountered across the experimental studies were whole in their form (Figure 11). Up to 36% of diatoms were classified as broken or fragmented at Stage 1, 32% at Stage 2, 43% at Stage 3, and 50% at Stage 4. Variation was observed across the three replicates examined at all stages of the experiment. For example, the distribution of fragmented diatoms at Stage 3 varied substantially with results of 43% (A), 14% (B), and 36% (C). A similar pattern was also observed in Stage 4 samples.

Figure 11. The distribution of whole and fragmented diatoms in the assemblage of each experimental sample (per cm2 of clothing analyzed).

Discussion

The two studies presented within this paper demonstrated the potential to use scanning electron microscopy for diatom analysis at various stages of a forensic investigation.

Study One

The results of the first study highlighted that the initial recovery of evidence by taping the clothing with an adhesive SEM stub, provides a valuable insight into the types of environmental traces available for analysis following transfer. Sampling via the “extraction” technique tested allowed for the screening of a larger surface area of a clothing garment, than has previously been considered in the collection of diatoms (Scott et al. 2014). When compared to the SEM analysis of a localized area (“embedded” samples), higher diatom numbers were consistently recorded in all extract samples (Figure 4). This technique may offer intelligence and guidance for further evidence collection and analysis, by mapping transfer “hotspots” within a clothing garment. Where obvious staining might be visible to the naked eye, analysis of a small subsample, through chemical extraction or in situ high powered microscopic examination, may be more beneficial in the first instance.

In all of the experimental samples analysed, diatoms were commonly observed in their natural form. A high number of intact diatom cells (i.e. two valves connected by girdle bands) were identified alongside diatom colonies attached to substrata (Figure 12).

Figure 12. SEM micrographs of diatoms recovered from extracted samples: A) Cocconeis pediculus (x950 magnification) observed attached to an unknown surface. Up to 9 diatom cells are observed in whole and fragmented form [Sample Cotton 1B]; B) Pennate diatom cell observed in girdle view (x5,500 magnification). In-depth species identification is limited due to a limited view of the valve face [Nylon 2B].

Most of the mechanical or chemical diatom preparation techniques described in the literature, frequently lead to the separation of the frustule into the two separate valves. This separation is useful for identification which is primarily based upon the ornamentation of the valve face. While the techniques described successfully allow for the observation of undamaged, mainly whole diatoms (Figure 5), this may also serve as a limitation for the accurate species-level identification of particulates using the SEM.

The SEM assessment of extracted surface samples highlights the successful use of electron microscopy as a screening tool that can act as a first step, to rapidly identify what types of freshwater trace evidence are present and whether further analysis is likely to be valuable. This efficiency is crucial for implementation in forensic practice where one of the main issues for environmental trace evidence examination is the time required for the preparation and analysis of evidential samples. Of the samples examined in this study, diatoms were the most consistently abundant organism, although green algae, mineral and pollen grains, and anthropogenic particulates were also present (Figures 6–7). Based upon this observation, further chemical preparation of samples may be recommended for the individual identification and analysis of the diatom species present (Scott et al. 2014). This highlights that the SEM offers an effective scanning method to assess the need for further (more time consuming or costly) analysis.

Although the main advantage provided by this approach is the immediate observation of evidence presence/abundance, the preservation of the organic and inorganic components of a sample for identification may also contribute exclusionary discrimination and promote a more detailed analysis. The quantity of evidence recovered during a forensic investigation is often microscopic, and so any collection or analytical techniques employed should endeavour to be as non-destructive as possible (Morgan and Bull 2014).

The value of pursuing several independent lines of enquiry has been documented in the forensic study of soils and sediments (Morgan and Bull 2007a), however no prior recommendations are outlined when considering freshwater environments. The preservation afforded by the SEM technique described, offers several forms of freshwater trace evidence for analysis, and contributes corroborative circumstantial information and exclusionary value for the accurate and robust interpretation of trace evidence in court.

Biological (plant) organisms were more commonly encountered in this study, although physical evidence types such as minerals and anthropogenic particulates were also frequently observed. In sample Cotton 1C, an unknown spherical particulate was identified and analysed using SEM-EDS (Figure 7). Elemental analysis indicated that the particulate was a glass bead, a characteristic component of road paints, implying that the source location could be in close proximity to a road or highway. Further discriminatory value can be discerned through the presence of salt dissolution on the surface of the particulate, which is a notable characteristic considering the sample site was brackish.

Finally, it should be noted that the quantitative and qualitative analysis of those diatoms in situ or embedded within the garment was often difficult due to the clothing surface characteristics or fibres obscuring the field of view. Although the extraction technique allowed for the clearer observation of several forms of evidence, the collection of samples may only have considered those particulates easily removed from the clothing surface. This has important implications when considering the forensic value and interpretation of an identified diatom assemblage using the SEM, as it is entirely feasible that some more adhered evidence may be retained within the evidential surface, and therefore not be present for analysis on the SEM stub.

Study Two

The results from the second study indicated that the diatoms transferred to clothing were preserved following various levels of exposure to fire, although the availability of particulates decreased over time. The SEM was able to distinguish diatom valves amongst the clothing fragments, providing a quantitative assessment even when the quality of evidence was partially compromised.

Despite an overall decrease in the number of diatoms identified over time exposed to fire, up to 641 valves (per cm^2 of material) continued to be recorded following the complete destruction of clothing to ash (Figure 9). The preservation of geoforensic indicators when exposed to fire and extreme temperatures has previously been examined in relation to the morphology and modification of quartz grain surface textures and pollen grains (Morgan et al. 2008, 2014a). Experimental studies highlighted that while quartz grains (primarily silicate) maintained a threshold of 1200°C before adaptation or destruction, pollen grains were a less resistant form of evidence. Despite the range of exposure times examined, it was not always possible to identify the three pollen morphologies studied following 30 seconds of heat at 700°C.

Although similar to pollen (in that both are biological plant microorganisms), the morphology of diatom valves in this study was unaffected by temperatures of up to 850°C with no modification or destruction observed (Figure 13). This corresponds with the temperature limits identified in the quartz study, implying that diatoms (composed primarily of silica) are preserved following a short exposure time to temperatures of up to 850°C.

Figure 13. A preserved pennate diatom (cf. Nitzschia spp.) observed in the burned clothing fragments at Stage 4 of the experiment (850x magnification).

The decline in the number of diatoms recovered at the various stages of this experiment may be attributed to the loss of a “stabilising” evidential surface (upon which to adhere), leading to the airborne transportation of diatoms. However, the continued recovery of diatoms, and the retention of their distinctive ornamentation for identification, has important implications during the investigative process. The preservation of particulates demonstrates that it is worthwhile to sample clothing which has undergone destructive acts for the collection and analysis of diatoms traces, even if there are delays in the search and recovery of evidence.

Analysis of the control water used to spike the samples provided an interesting insight into the background distribution of centric and pennate diatoms; and the value of each class for the forensic comparison and exclusion of evidential samples (Figure 8). Although centric diatoms were the most abundant, only two genera were identified: Melosira varians was common to all control and experimental samples, whilst Stephanodiscus spp. was less frequently encountered. In comparison, pennate diatoms were less abundant but more diverse in the genera identified (Figure 8). The preservation of both groups in the experimental samples adds value for crime reconstruction. Whilst the presence of Melosira varians in abundance is indicative of a flowing environment, the diversity of pennate taxa adds further discriminatory value for the exclusion of forensic samples. Consideration of both the ubiquitous and the more rare components of a sample can provide meaningful intelligence in a range of environmental and forensic investigations (Morgan and Bull 2007a), including the analysis of diatom trace evidence.

Assessment of the control site diatom assemblage offered a useful reference sample for comparison, although no in-depth species identification was performed. Whilst the decay in the availability of both pennate and centric diatoms varied slightly, both groups were identified at similar rates (17–20%) at Stage 4 of the experiment (Figure 10). The presence of Melosira varians continued to be identified providing distinctive environmental information, however only one genus of pennate diatom was recorded. This suggests that although such taxa are retained upon clothing, they may be less diverse in their species composition over time, reducing the exclusionary value of a forensic diatom sample over time.

SEM analysis offers a valuable initial insight into the extent of diatom preservation following the destruction of a recipient transfer surface. While it is clear that diatoms are still present in varying quantities, the reduction of diatoms within a system is perhaps less well understood. Crucially, this study contributes data to empirically support the collection of damaged (or fragments of) clothing materials as evidence over various investigative timescales, in order to try and recover environmental traces for forensic comparison. Future work might also consider the use of light microscopy, to further examine the preservation dynamics of diatoms and to compare the analytical capability with that of the SEM.

General discussion

This paper demonstrates the value of adopting an empirical approach to develop the evidence bases needed to support the robust and appropriate interpretation and presentation of freshwater trace evidence in court.

The two studies presented in this paper demonstrate the benefits of using electron microscopy for the analysis of diatoms and alternative freshwater indicators at various stages of an investigation. There appears to be value for identifying what (if any) evidence is present on a recipient clothing sample, in order to inform the best approach for further analysis, and in the assessment of any remaining particulates following attempts to destroy clothing. The data generated provides an initial evidence base for establishing recommendations guiding the appropriate sampling approach of evidential clothing items contacted with freshwater crime scenes, and provides insights into the preservation of diatoms when attempts have been made to destroy evidence by burning.

By determining the initial and continued presence of environmental materials on clothing, recommendations can be made for its collection and analysis (e.g. in situ or extracted examination) and limitations for interpretation outlined. For example, the diversity and variability of a diatom assemblage at a given site is one of the main traits offering value for forensic application (Cameron 2004). The results of the studies presented here highlight that there may be spatio-temporal variation in the availability of a forensic sample through the selective extraction of surficial materials for analysis (study one) or in the selective persistence of centric and pennate species over time (study two). This has important consequences for the geoforensic comparison and exclusionary value of known samples, which often relies upon examination of the ubiquitous and the rare components of a specimen. Decline in the species diversity in study two was noted based only upon the identification of diatoms to genus level. The identification of diatoms (and other freshwater algae) to species-level may contribute the additional research required to fully understand the interpretative constraints identified following the persistence of diatom evidence during and after a criminal event in a freshwater environment.

High resolution examination of forensic diatom samples using the SEM, has only previously been used to support and corroborate data generated through assessment using light microscopy (Scott et al. 2014). This study highlighted that SEM has the potential to be used independently, and the data quantified for comparison. The value of the SEM for diatom analysis is twofold: primarily, it allows for a non destructive, initial quantitative assessment of trace material (up to a few microns); and secondly it allows for qualitative consideration of a sample surface at high resolution (Figure 1).

Since the ornamentation of the diatom frustule is so complex, detailed observation at higher magnification substantially aids in species identification. The empirical studies presented here highlight the value of such an in-depth approach, however the natural characteristics of the samples assessed in the first study also acted as a limitation. Collecting adhered materials directly from the surface of the clothing, without prior treatment, resulted in the retention of natural diatom characteristics, such as the intact cell, but restricted the feasibility of more detailed identification based upon valve morphology. Chemical methods such as the H2O2 digestion of environmental and forensic samples described throughout the literature are therefore deemed more appropriate for the individual assessment and identification of diatom populations (Scott et al. 2014). The SEM method described does however, provide a valuable initial screening approach that offers an overall assessment of relatively large areas of clothing to indicate what trace evidence may be present and can inform subsequent collection and analysis of trace evidence from an exhibit.

In order to appropriately direct collection and analytical techniques and consider the evidential significance of transferred diatom and freshwater evidence, it is important to acknowledge the context of an individual case. Factors such as the type, length, and extent of transfer, and the characteristics of a recipient surface affects the amount and quality of evidence available for consideration. The most pertinent example of this within freshwater environments is the distinction between an initial contact with a benthic (bottom/surface sediment) or planktonic (suspended water) environment, which would likely influence the composition of those organisms transferred to an evidential sample. This has important consequences for crime reconstruction. The collection of representative control samples at the crime scene, or alibi verification site, is highly recommended to ensure a comparator is available for the exclusion of any evidential samples later recovered. These samples will also provide an early indication on the types of freshwater particulates likely to be recovered as trace evidence throughout an investigation.

The forensic study of diatoms as trace evidence indicators is gathering impetus in the research literature, but further empirical study is needed to establish and broaden the range of circumstances in which particulates are actively sought as evidence in casework. Experimental consideration of the persistence of diatoms over time or the variation in their transfer to alternative evidential surfaces, for example, has the potential to contribute valuable knowledge within the field of forensic limnology.

Conclusions

Current forensic geoscience investigation is primarily concerned with the independent analyses of terrestrial soil and sediment samples, however, there is a need for a similar approach within the context of freshwater trace evidence analysis. This paper demonstrates the value of empirical research, supported by existing case work practice, for the development of an evidence base for the utilization of diatom trace evidence in forensic limnology. The pursuit of experimental studies, such as those presented here, is crucial in creating the empirical evidence base necessary for the application and development of new collection and analytical procedures, and to enhance the understanding of freshwater trace evidence dynamics for legal interpretation. Specifically:

- Diatom analysis is widely utilized in a range of forensic contexts, and contributes important circumstantial intelligence following its transfer from a crime scene to a perpetrator or victim;
- The study of alternative algal groups has the potential to provide a comprehensive ecological account of a freshwater environment for use in crime reconstruction;
- SEM analysis provides a valuable tool for the quantifiable and multidisciplinary assessment of aquatic trace evidence following transfer;
- High resolution optical examination offers valuable insight into the availability and quality of adhered traces at various stages of the investigative process—from the initial screening of an evidential surface to the long term preservation of evidence;
- Diatoms can be preserved on clothing even after attempts have been made to destroy evidence through burning.

It is therefore worthwhile to sample degraded clothing items to recover any remaining aquatic trace evidence that may still be present. Whilst experimental research provides important information pertaining to the collection, analysis, and interpretation of environmental forensic evidence, it is also important to recognize the value of expert knowledge. Nevertheless, empirical evidence bases do offer a basis for the inference of the weight and significance of encountered evidence. The value of such foundations must be acknowledged, particularly given the trajectory of requiring such evidences bases to deem trace evidence admissible in court (Law Commission Report 2011; Annual Report of the Government Chief Scientific Adviser 2015). The value of freshwater indicators is yet to be fully realized and established within the broad field of trace evidence analysis. Given the complexity of aquatic environments and their associated physical, chemical, and ecological variabilities, there is significant potential for aquatic trace evidence to offer valuable circumstantial intelligence for the comparison and exclusion of samples or in the profiling of an unknown location. Finally, the transfer and persistence of freshwater diatoms and other algal trace indicators on clothing, indicates that there is great potential for the recovery of evidence during crime reconstruction in aquatic environments.

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