



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



Kirstie R. Scott^{a,b,c,*}, Ruth M. Morgan^{a,b}, Vivienne J. Jones^c, Nigel G. Cameron^c

^a Department of Security and Crime Science, UCL, 35 Tavistock Square, London WC1H 9EZ, UK

^b Centre for the Forensic Sciences, UCL, 35 Tavistock Square, London WC1H 9EZ, UK

^c Environmental Change Research Centre, UCL, Pearson Building, Gower Street, London WC1E 6BT, UK

ARTICLE INFO

Article history:

Received 7 February 2014

Received in revised form 12 May 2014

Accepted 14 May 2014

Available online 23 May 2014

Keywords:

Forensic geoscience

Diatoms

Trace evidence

Transfer

Collection procedure

Soil Collection procedure

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 H₂O₂ 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 H₂O₂ 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 H₂O₂ 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.

© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0/>).

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 (SiO₂) is resistant to decay and retains diagnostic features enabling species identification and forensic comparison [22]. Furthermore, the

* Corresponding author. Tel.: +44 20 3108 3206.

E-mail addresses: k.scott.12@ucl.ac.uk (K.R. Scott), ruth.morgan@ucl.ac.uk (R.M. Morgan), vivienne.jones@ucl.ac.uk (V.J. Jones), n.cameron@ucl.ac.uk (N.G. Cameron).

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 cm² 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 cm² 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

submerged in the stream water following extensive chemical (H) and mechanical treatment (RE, RW). Those t-shirt samples submerged for 3 min and 24 h were adhered to S.E.M. stubs, gold coated and examined at 150,000× magnification (Jeol JSM-6480LV).

Diatoms were observed in the weave of the clothing and documented according to their main characteristics: size, shape and whole/fragmented form. Diatoms were counted and recorded per mm² of t-shirt.

3. Results

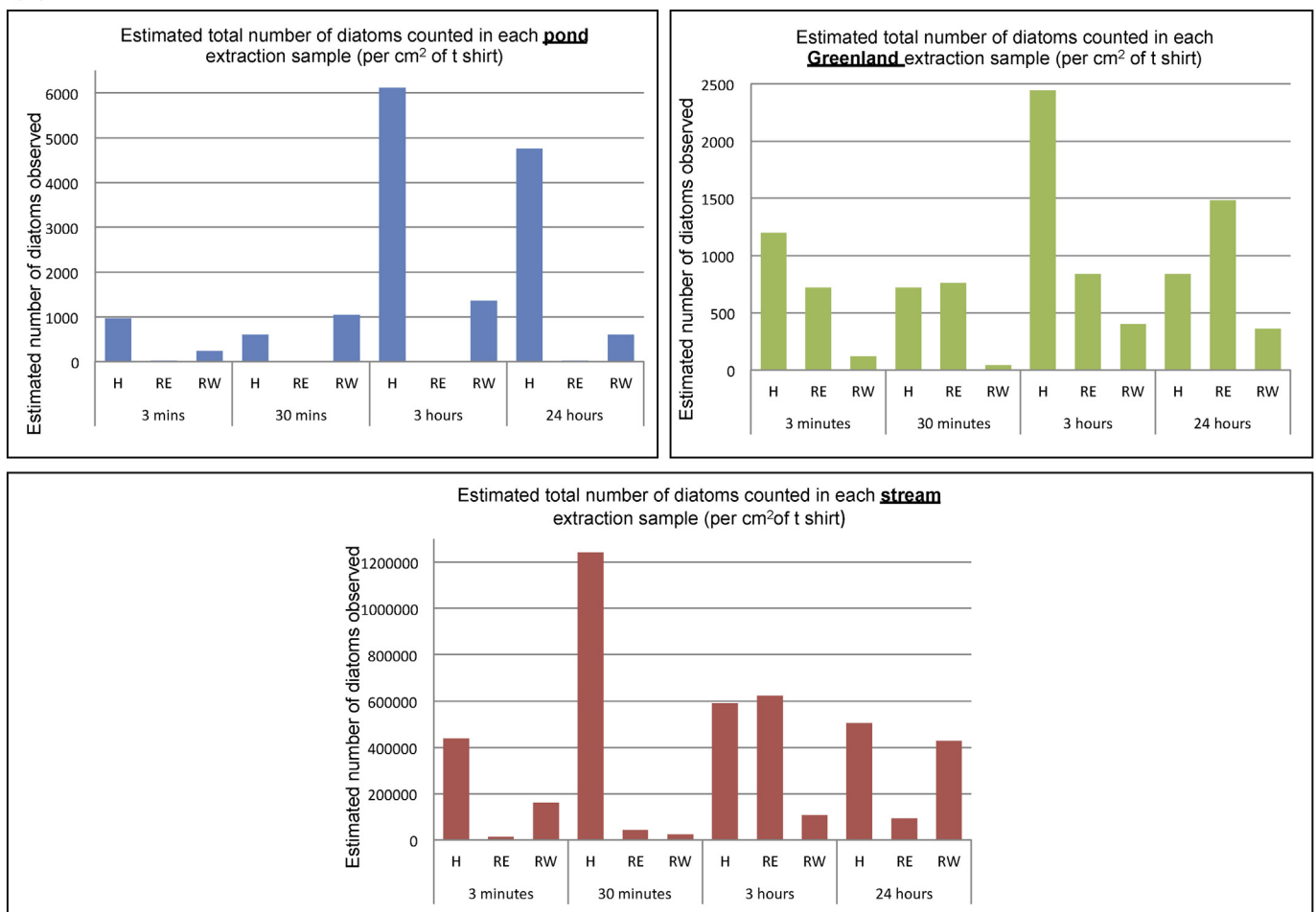
3.1. Aquatic extraction

The estimated total number of diatom valves recovered after each extraction technique is shown in Fig. 1. A greater number of diatom particulates were recorded in the stream control sample when compared to the pond and Greenland sites which was reflected in the experimental samples. Over 12,000 valves per cm² of t-shirt were observed in all experimental stream samples,

compared to fewer than 1000 valves in the majority of the pond and Greenland sites. A greater proportion of diatoms were recovered after H₂O₂ extraction in all samples (Fig. 2). The diatom yield from RE and RW extraction varied amongst sites, with a higher proportion of diatoms recovered after ethanol rinsing in Greenland samples (RE [383%]) and water in pond samples (RW [206%]). The majority of diatoms observed in all t-shirt samples were extracted following 3 h of submersion (Fig. 3). Though still relatively high, diatom counts after 24 h decreased marginally. Counts were lower after shorter submersion intervals.

Species richness (the total number of diatom taxa identified in a sample) was higher in stream and Greenland sites when compared to the pond. All control samples identified distinctive assemblages, composed of both abundant species and exotic markers. For example in the stream control site, *Melosira varians* was observed in abundance whereas *Gomphonema truncatum* was relatively sparse. Composition data for the t-shirt samples reflected this trend. Correspondence analysis plots highlight the similarity of experimental and control samples in their species assemblage. The closer sample points are in proximity displays greater similarity

(A)



(B)

	Estimated total diatom count: Control Sample 1	Estimated total diatom count: Control Sample 2
Greenland	286	58
Pond	328	1472
Stream	3,176,000	-

Fig. 1. (A) The estimated total number of diatom valves (per cm² of t-shirt) recovered from each aquatic site (pond, Greenland, stream) following three extraction techniques: rinsing with ethanol (RE), rinsing with water (RW), and H₂O₂ submersion (H). Each extraction method was tested on four t-shirt samples immersed in the water site for various time intervals (3 min, 30 min, 3 h, and 24 h). (B) The estimated total number of diatoms (per ml of water) recorded in replicate comparative control samples.

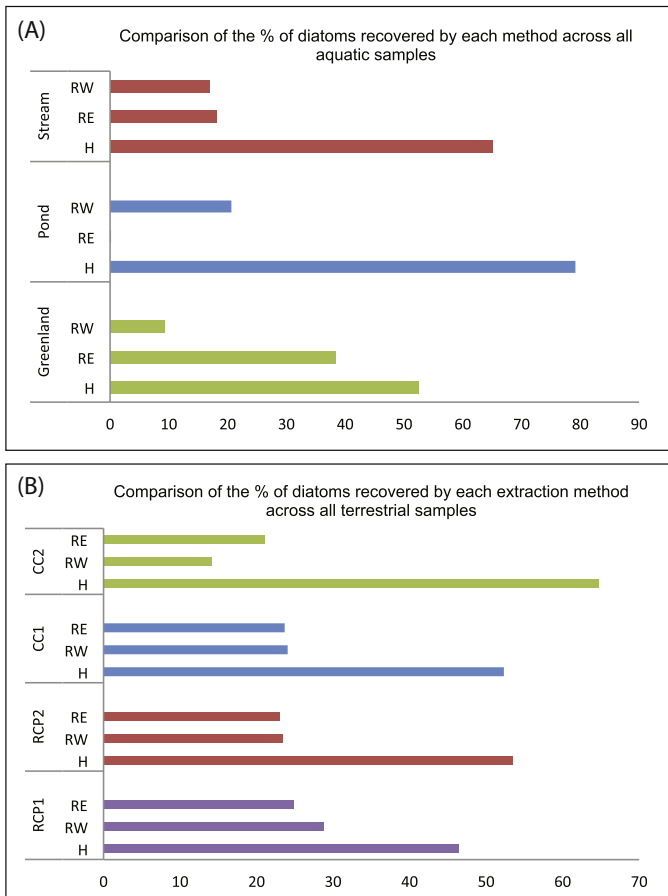


Fig. 2. (A) Comparison of the total diatom yield (%) recovered by each method across all aquatic samples. Results highlighted the effectiveness of the H₂O₂ treatment in recovering a greater proportion of diatoms when compared to RE and RW. (B) Comparison of the total diatom yield (%) recovered by each method across all terrestrial samples. Results again highlighted the effectiveness of the H₂O₂ method in extracting over 50% of all diatoms detected in the samples. RE and RW recorded greater relative success in the recovery of diatoms from soil samples than in aquatic study.

than to those samples further apart (Fig. 4). In the stream samples, all extraction methods exhibit affinity to the control site although greater similarity is observed in all four of the H₂O₂ samples. The Greenland and pond t-shirt samples display greater variation amongst experimental samples, with greater similarity witnessed in the species composition of diatoms extracted by H₂O₂ submersion. This is an important finding as it suggests that H₂O₂ samples consistently resemble the greatest similarity to the relevant control site.

3.2. Terrestrial extraction

Large numbers of diatom valves per cm² of clothing were extracted from the four soil sites (Fig. 5). The estimated diatom count recorded across the terrestrial sites was variable which was reflected in the transferred experimental diatom count. Over 1000 diatom valves were recorded per cm² of t-shirt in the majority of experimental Ravenscourt Park samples, with higher values approaching approximately 200,000 diatoms per cm² across both Clapham Common sites. Each extraction method was repeated three times, with large variations observed in the estimated total number of diatom valves recorded within each method. For example, diatom counts ranged from c.1710 to 4240 valves/cm² in RCP1 samples following H₂O₂ extraction, c.1320–2120 valves/cm² following RW and c.80–2160 valves/cm² following RE. H₂O₂ extraction recovered

the highest diatom yield in all four scenarios with an estimated 4240 particulates per cm² of clothing compared to 2120/cm² (RW) and 80/cm² (RE). Of all the diatoms recovered from the t-shirt samples, over 45% at all four sites were attributed to extraction by H₂O₂ (Fig. 2). The proportion yielded from RW and RE was lower, but consistent amongst the two techniques.

Species richness was lower in terrestrial samples when compared to the aquatic environments (Fig. 6). While each aquatic site provided a specific distinctive assemblage of abundant genera and exotic indicators, the soil sites shared some common aerial diatom species. *Hantzschia amphioxys* was found in abundance (>40%) in all four locations sampled. H₂O₂ samples display marginally greater species richness in contrast to RE and RW. Some less abundant species found at the control sites were not always observed in the corresponding experimental samples. *Cocconeis* was identified in trace amounts in the RCP2 control sample; a trend only reflected in three experimental samples (RW1, H1, and H2). The similarity of experimental and control sample species assemblage is highlighted in Fig. 7. In Clapham Common samples, similarity to control sample species composition varies spatially with correspondence highlighted amongst H₂O₂ samples in CC1 and RE samples in CC2. A similar trend is apparent in Ravenscourt Park samples where H₂O₂ and RE samples display greater similarity to control samples.

3.3. S.E.M. observation

A greater number of diatom particulates were found to persist on those experimental samples treated with ethanol (RE) following both 3 min (23 per mm² of clothing) and 24 h submersion (35 per mm² of clothing) in the stream (Fig. 8). In both instances, fewer diatoms were observed in the H₂O₂ treated samples. A greater number of valves were recorded in the 24 h samples. Those diatoms observed were primarily characterised as >5 μm in size, whole, and pennate in their form. An example of multiple diatom frustules embedded in the weave of the cotton t-shirt is provided in Fig. 9.

4. Discussion

4.1. Aquatic extraction

Relatively high numbers of diatom valves were obtained in all three environments sampled, with diatoms found on almost all the

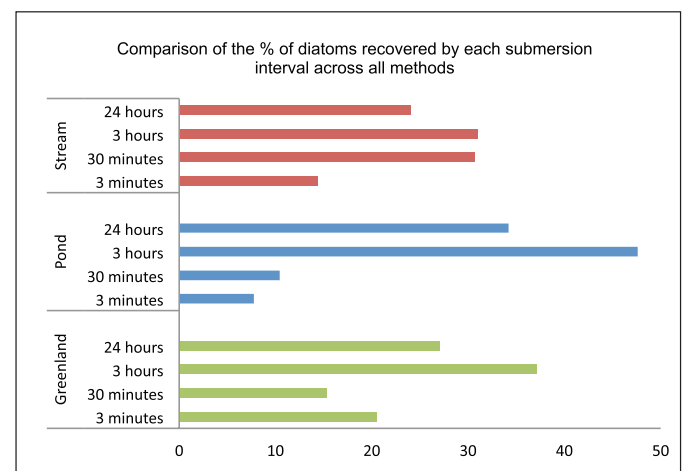


Fig. 3. Comparison of the total diatom yield (%) recovered following each submersion interval across stream, pond, and Greenland sites. In all three environments sampled, results showed that a greater proportion of diatoms were found to transfer following 3 h of submersion.

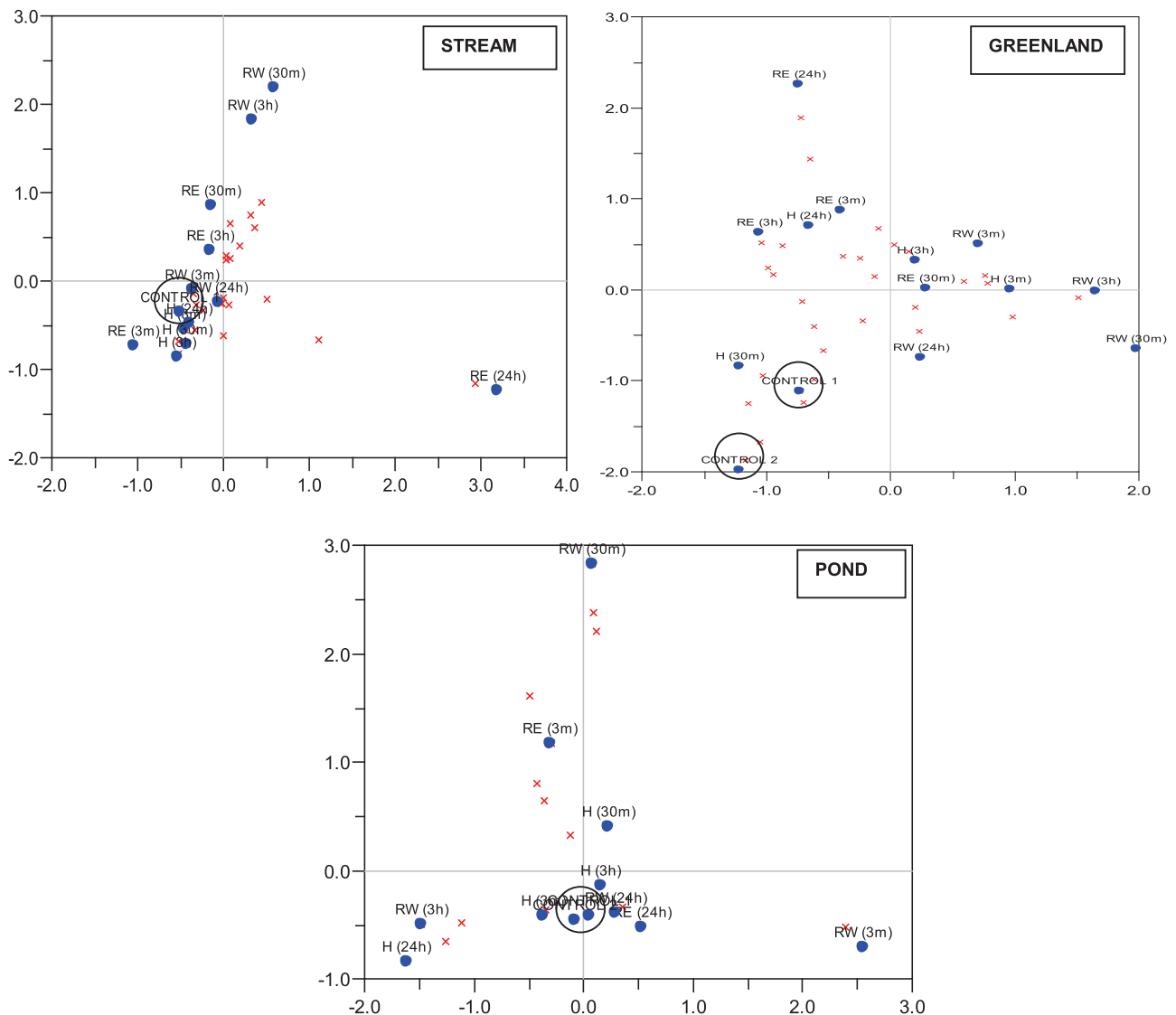


Fig. 4. Correspondence analyses of aquatic species highlighting the similarity of experimental RE, RW, and H₂O₂ sample composition alongside control samples. Control samples are identified by **o** with individual diatom species identified by **x**. Control samples are circled.

experimental samples and only two nil results noted. The highest estimated diatom valve counts were recorded in the experimental stream samples with up to c.1,240,920 valves/cm² of t-shirt identified following H₂O₂ extraction. Such results were obtained from analysis on only small (1 cm²) sections of t-shirt, highlighting the great potential for diatom analysis over both small and larger evidential surfaces areas. The extent of diatom transfers as forensic evidence is related to the existing diatom population in the source location. These results suggest the need for the investigator to recognise and collect reference water samples at crime scenes for the potential comparison of samples taken from exhibits pertinent to the case at a later date.

The H₂O₂ treatment displayed the highest estimated diatom yield for all experimental aquatic samples. Direct comparison of the three techniques adopted shows that over 50% of the diatoms identified across all four time series were recovered by H₂O₂ treatment and RE and RW yielded fewer diatoms. For example, in the pond samples c.6120 valves/cm² were recorded in H₂O₂ samples submerged for 3 h compared with 1360 per cm² for RW and 0 for RE. Such low (and nil) values following RW and RE may indicate that these extraction methods may not be viable for the forensic comparison and exclusion of samples. The efficacy of the

RE and RW methods differ between environments, although the H₂O₂ results are consistent across the three sites. These results highlight the sensitivity of each extraction method and the need to adopt an efficient treatment in order to yield the most representative sample possible for comparison. It is also important to account for both the abundant and the more rare components of a sample. Such aspects of a sample are invaluable in the exclusion of a sample or the profiling of an unknown environment. The results of this study therefore indicate that H₂O₂ extraction is the most efficient technique for use in the collection of diatoms as evidence in aquatic contexts for subsequent forensic analysis.

Higher diatom valve counts were recorded from samples that had been exposed to longer submersion intervals, with the highest number observed in those 3 h immersion samples. For instance, of all diatoms recovered from Greenland t-shirt samples, 37% were identified after 3 h, compared to 27% (24 h), 15% (30 min), and 20% (3 min) (Fig. 3). Importantly, a moderate proportion of diatoms were recovered following brief contact between the t-shirt and the water body. Of all the diatoms recovered from the stream t-shirt samples, 14% were recovered following 3 min of submersion (c.612,000 valves/cm² of t-shirt). The recovery of diatoms following various time periods of exposure to aquatic environments is of

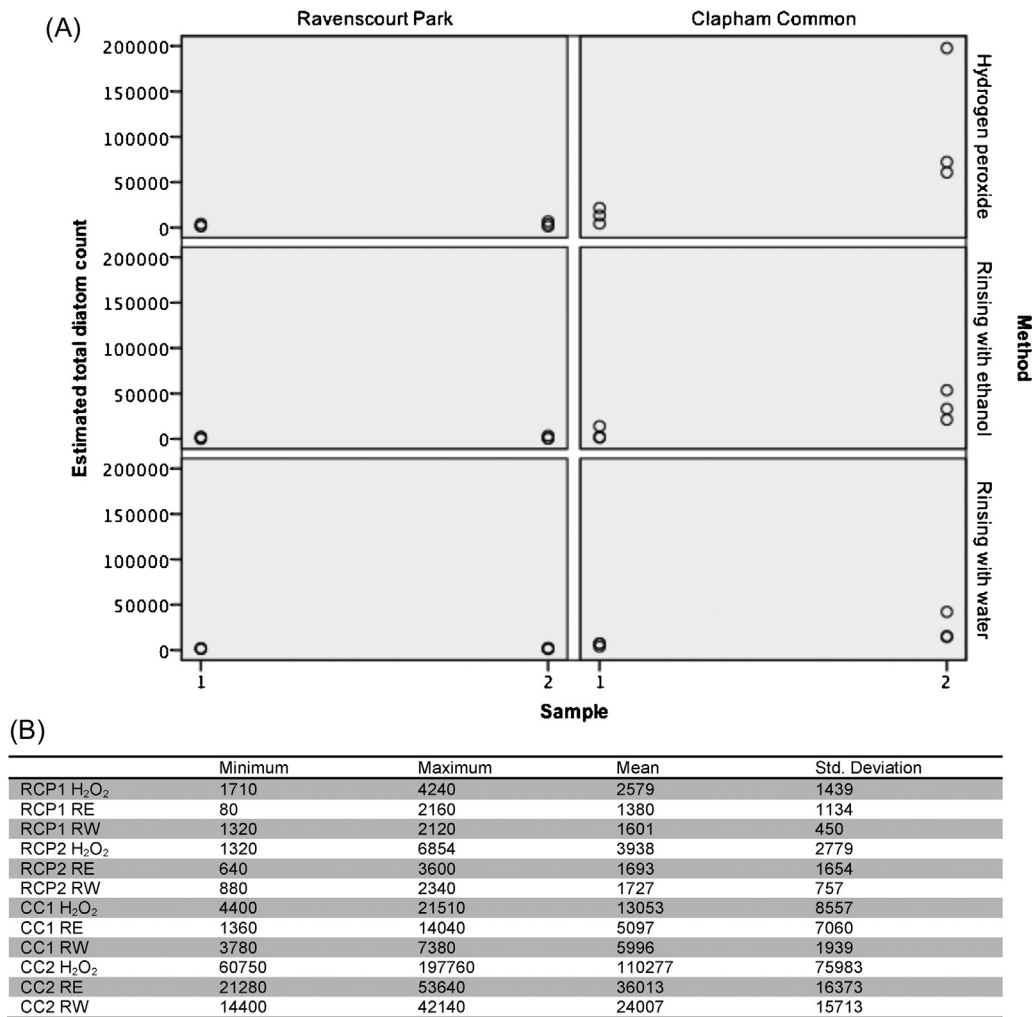


Fig. 5. (A) The estimated total number of diatom valves (per cm² of t-shirt) recovered from each terrestrial site following three extraction techniques: rinsing with ethanol (RE), rinsing with water (RW), and H₂O₂ submersion (H). Each method was repeated three times for each sample. (B) The mean and standard deviation of the estimated total diatom count within each terrestrial sample.

great utility to forensic geoscience. Criminal investigation often involves the examination of evidence that may have been stationary within a water body for an extended time period (for example, the deposition of a murder weapon), as well as the analysis of evidence in short-term contact with water (e.g. the perpetrator leaving a crime scene). These results provide interesting evidence to illustrate that only short contact between clothing and a water body can generate significant transfers of diatom traces as evidence, which should be recognised and collected for analysis in criminal investigation. Furthermore, in those environments where diatoms are scarce, it is important to ensure the technique used for the collection of evidence is optimal.

In this study, all diatoms in a sample were identified and counted in order to account for the appropriate representation of species richness, considering both the abundant and the more rare taxa. Those aquatic sites where more individual valves were counted also recorded a higher number of diatom species as reflected in the experimental sample species composition. The samples exposed to the pond were composed of 10 species/cm² of t-shirt while 28 species/cm² of t-shirt were identified in Greenland experimental samples. The diversity of more abundant sites offers great value to forensic geoscience and the pursuit of profiling an unknown environment as well as the comparison and exclusion of samples from a common source. Certain diatom species across the three locations sampled tend to be associated with distinct habitats, which

may be reflected in their forensic application. *Melosira varians* is observed in high proportions in the stream samples but is absent from both pond and Greenland sites. *Melosira varians* is a diatom species indicative of running water [59], and in this context its abundance could be used explicitly to exclude a suspect from a stationary water body such as a pond, lake, or reservoir.

Importantly, the species identified in the experimental samples reflects both the distribution of the common and the more rare diatoms in the relevant control sample. In stream samples, *Rhoicosphenia curvata* and *Type 27* account for <1% in the majority of samples, while *Synedra ulna* represents over 10% of the total composition. The presence of both the abundant and unusual components of a sample is important for forensic interpretation. A number of species were identified in experimental samples, which were not previously observed in the control site. *Gomphonema sp.1* was observed in several pond transfer samples yet was unrecorded in the reference site, highlighting the importance of collecting multiple control samples for comparison.

These findings suggest that careful consideration must be given when profiling or excluding an area based upon the analysis of extracted diatom communities. Those environments most abundant in their habitual diatom population report higher consistencies in their total number of species. In aquatic contexts, H₂O₂ treatment is recommended as the most effective in collecting a representative sample for analysis.

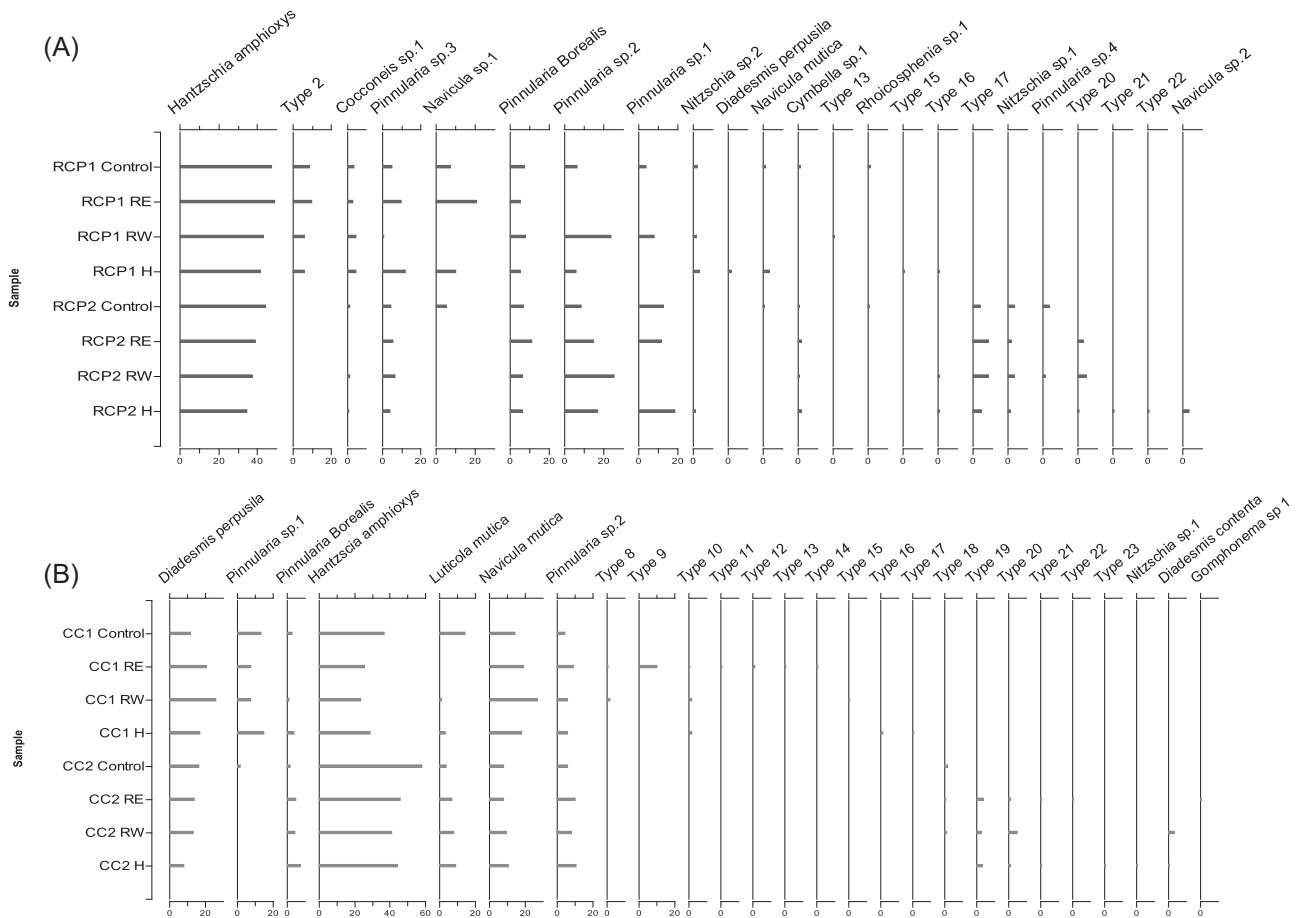


Fig. 6. Relative species composition of t-shirt extraction samples and soil control samples for terrestrial sites: Ravenscourt Park (A) and Clapham Common (B).

4.2. Terrestrial extraction

The estimated total diatom valve count in the four soil locations sampled varied, reflected in the total number of diatoms transferred to the t-shirts following contact. While the most transferred diatoms were recovered in the stream samples (c.1,240,920 diatoms/cm² of t-shirt), the soil sites from Clapham Common also displayed high values of up to 197,700 valves/cm² of t-shirt following H₂O₂ immersion. These findings highlight that diatom traces are, in select cases, relatively abundant in soils which enhances their potential for application in forensic geoscience. Soils and sediments are commonly recovered from crime scenes [4] and trace analysis can provide important environmental information. The high number of diatoms collected contributes important empirical data to suggest that ecological analysis of soil diatom communities has the potential to provide an independent line of enquiry in the forensic comparison of soil samples.

Few diatom particulates were recorded in some Ravenscourt Park samples (80 valves/cm² of clothing post RE) again displaying the need to ensure an optimal extraction technique is used for geoforensic comparison. Variation in the diatom communities was observed over small spatial areas. Diatom counts in the two Clapham Common sites metres apart were variable. In CC1 sites, values approached 13,000 valves/cm² following H₂O₂ extraction, whereas this number was exceeded in all CC2 samples. This change highlights that control samples should be appropriately collected to ensure that a representative sample is available for comparative analysis. It is well known that diatom communities change temporally [21]; however, the forensic practitioner should also be knowledgeable in such discreet spatial diversities within a crime scene.

The H₂O₂ treatment displayed the highest estimated diatom yield in all four soil habitats sampled. The success of the H₂O₂ treatment is less pronounced than in aquatic environments with large variability observed in the total number of diatom valves recorded across the three experimental runs. For example, the standard deviation in Ravenscourt Park 1 H₂O₂ samples was recorded at 1439.26, compared with 1133.54 (RE) and 449.72 (RW). The same trend is also reflected in the second Ravenscourt Park and both Clapham Common sites. These findings indicate the need for multiple experimental samples to be assessed when pursuing H₂O₂ treatment for the extraction of the highest diatom numbers. Furthermore, while the H₂O₂ treatment extracted 52–79% of the estimated total diatoms in aquatic contexts, values were below 50% in three of the four soil sites. These results indicate that although H₂O₂ extraction remains the most effective, RE and RW observe more success in extracting soil diatoms from clothing than in aquatic contexts. The success of the H₂O₂ technique in aquatic scenarios may be explained by the absorption of diatoms into the weave of the fabric. H₂O₂ extraction provides a more effective chemical reaction agitating the embedded diatoms for collection, when compared to RE and RW. In the terrestrial samples, diatoms are likely to remain on the clothing surface. The terrestrial diatoms are therefore more easily extracted with less vigorous methods (e.g. RE and RW) resulting in less of a difference in the diatoms recovered from the clothing between the three methods.

The four soil sites examined all displayed lower species richness when compared to aquatic sites. The total number of diatom taxa recovered in control samples ranged from 9 (CC2) to 15 (RCP2) species in a 2 g sample, while aquatic diatom species ranged from 10 (pond) to 28 (Greenland). Notwithstanding the

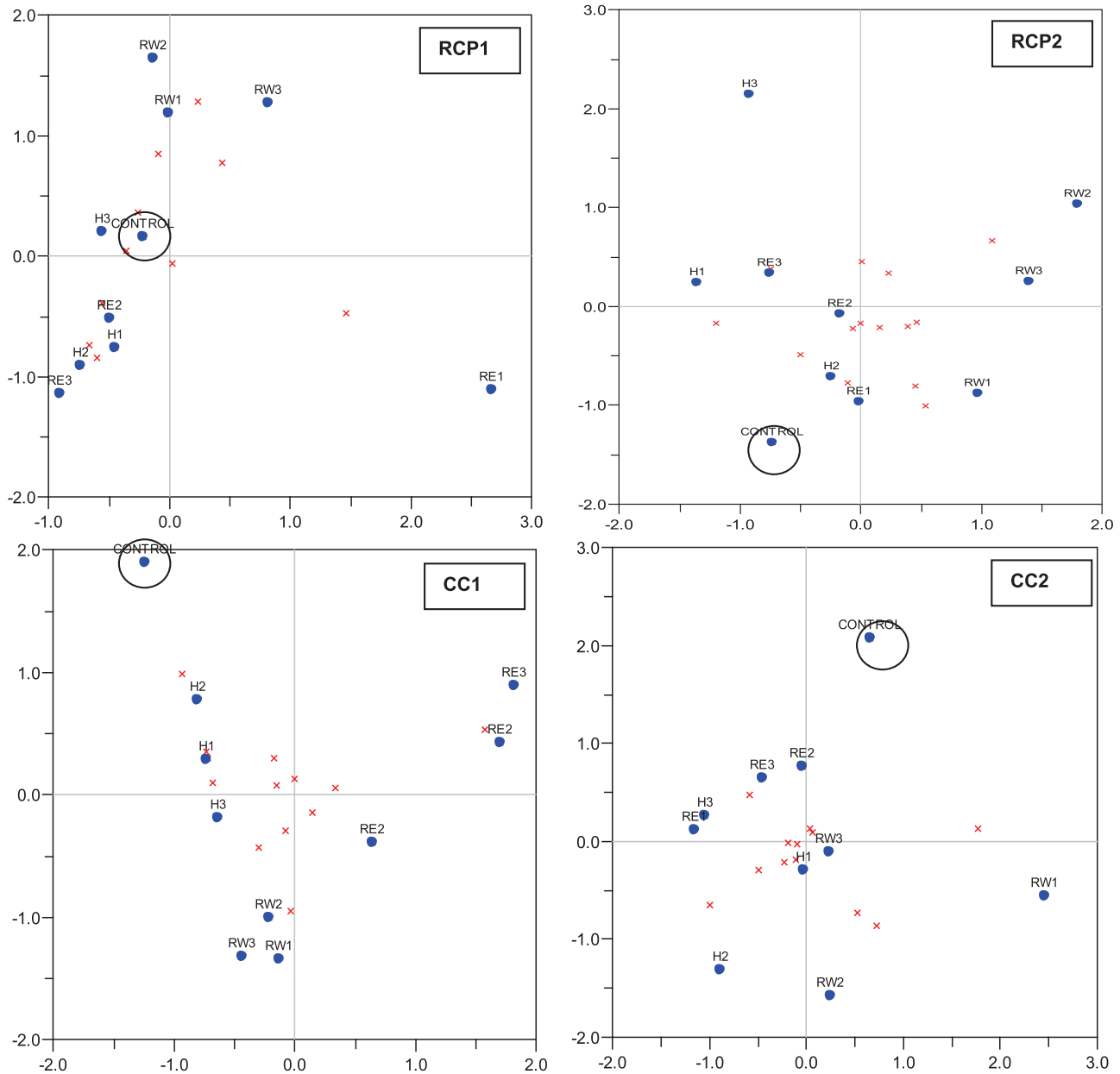


Fig. 7. Correspondence analyses of terrestrial species highlighting the similarity of experimental RE, RW and H₂O₂ sample composition alongside control samples. Samples are identified by **o** with individual diatom species identified by **x**. Control samples are circled.

reduced number of species, soil diatoms can still provide distinctive characteristics that may be valuable in profiling an environment. Certain taxa were found in abundance in all four soil sites sampled including *Pinnularia borealis*, *Hantzschia amphioxys*, and *Luticola mutica*. The presence of such species may restrict the function of diatom analysis in 'compare and exclude' forensic investigation, to that of a corroborative role alongside alternative environmental indicator techniques such as palynology and elemental composition.

The species identified in the control sites are well represented in the experimental sites. For example, *Hantzschia amphioxys* accounts for approximately 60% of the CC2 control site sample and is also observed in abundances of 40–60% in the experimental samples. *Diademesis perpusila* and *Luticola mutica* are observed in similar volumes in CC1. Reflecting the patterns identified in the aquatic samples, some rare species are recorded in transfer samples, which have not been identified in the control sites. It is therefore important that future research considers the preparation

of multiple control samples in order to account for all species which may be detected in the relevant transfer sample.

Although the H₂O₂ method yields the highest number of diatoms in both Ravenscourt Park locations sampled, not all diatom species identified from the control sites are represented. The presence of more exotic diatom species adds exclusionary value in the distinctive nature of environments. While *Pinnularia sp.1* and *Rhoicosphenia sp.1* are found in trace levels in the control site, neither is witnessed in the samples following H₂O₂ and RE treatment. This suggests that not all terrestrial diatoms are extracted equally with the methods adopted in this study.

Interestingly, although greater diatom valve counts were recorded at the Clapham Common sites, greater variation is observed in the species richness comparisons between control and experimental sites. While less similarity exists between the experimental and control samples than recorded in the aquatic sites; there is visual similarity within the techniques tested. In the first Clapham Common site, clusters of data represent the

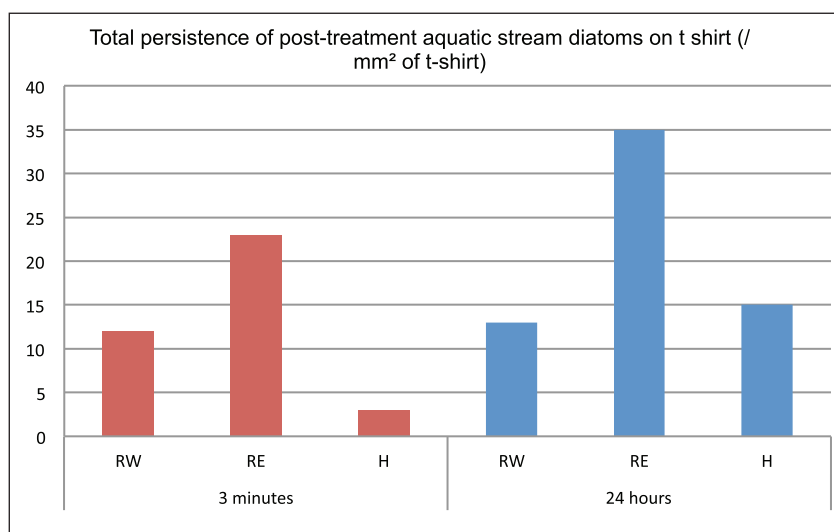


Fig. 8. Comparison of the total number of diatom valves (per mm² of clothing) retained on post-treatment stream clothing samples following 3 min and 24 h submersion. S.E. M. examination confirmed results which highlighted H₂O₂ as the most effective treatment for the analysis of clothing garments.

reproducibility of each technique— RE, RW, and H₂O₂ submersion (Fig. 7a). This indicates that the species composition is reproducible within each method tested, while large discrepancies exist in the initial total diatom yield.

4.3. S.E.M. Analysis

The examination of treated clothing under the S.E.M. provided a valuable insight into the dynamics and persistence of diatom particulates following chemical and mechanical extraction procedures. As observed in Fig. 8, the number of aquatic stream diatoms detected on t-shirts immersed for 3 min and 24 h was highest following ethanol rinsing and lowest following H₂O₂ submersion. For example, 3 valves/mm² of t-shirt were identified in 3 min H₂O₂ samples compared to 23 valves/mm² of t-shirt following the RE treatment. This contributes to the finding that RE recovered the fewest diatoms of all three methods following both 3 min and 24 h submersion. The retention of diatoms in the weave of the fabric implies that not all diatoms are extracted for comparison. The low nature of diatom retention in H₂O₂ samples adds weight to the

effectiveness of the method in yielding an optimal sample for analysis.

The presence of diatoms in the weave of fabric provides a preliminary insight into the persistence of particulates following transfer and collection procedure. These results imply the utility of forensic diatom analysis over longer temporal intervals. In forensic reality there is often a delay between the transfer and subsequent collection of evidence as clothing may be seized and sampled days, weeks, and even months after the initial criminal event. While the persistence of various types of physical evidence has been considered within the relevant literature [48,60–63], no published experimental research currently exists which considers the temporal decay of diatoms from clothing. Understanding the persistence of evidence is vital to geoforensic practice and the results of this study indicate that diatoms are retained on clothing and may therefore be useful long-term trace evidence indicators.

4.4. Synthesis

In all of the scenarios explored in this study, the examination of t-shirt samples treated by H₂O₂ consistently extracted the highest estimated diatom counts and those assemblages that most closely resembled the relevant control site species richness. The efficacy of the H₂O₂ method is further consolidated with the results of S.E.M. analysis which found lower rates of diatom retention within the weave of the fabric when compared to RE and RW. Diatoms were found to persist in high numbers following 24 h of RE/RW treatment although less consistency was observed across both aquatic and terrestrial t-shirt samples.

Practically, the H₂O₂ method was noted as the quickest of the three techniques tested with diatoms recovered in abundance following only 3 h of treatment. The potential for the oxidisation of organic clothing samples in H₂O₂ solution requires careful observation to ensure that evidential surfaces are not compromised. All three treatments tested were non-destructive allowing for the retention of clothing for future independent analyses.

5. Conclusion

This study has provided an important first step towards understanding the transfer dynamics of diatoms as evidence in forensic geoscience investigations, and identified the methods

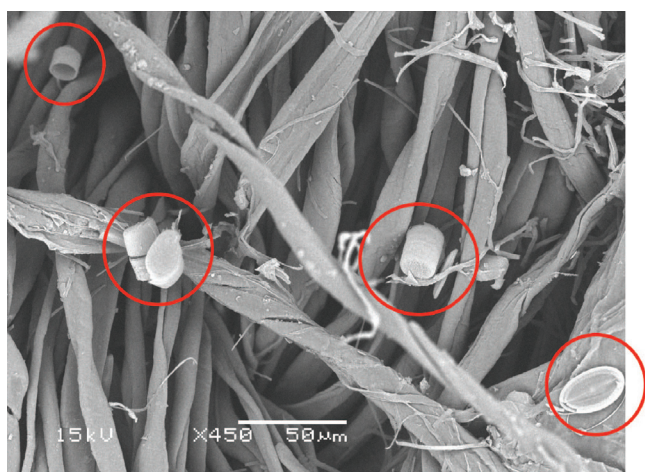


Fig. 9. S.E.M. image (×450 magnification) highlighting the retention of stream diatom frustules in the weave of the t-shirt fabric following RE treatment. Diatoms were observed in both valve and girdle view. Diatoms circled are identified as *Melosira varians* and *Cocconeis* sp.

appropriate for their collection from clothing. Diatoms can be used as circumstantial evidence in those crime scenes involving both water and soil evidence, although species richness is determined to be more distinctive within aquatic sites. Of the three methods examined, the H₂O₂ procedure is recommended as the most effective in yielding both the highest diatom count and most representative species richness data for comparison from clothing samples.

The findings of this research observe spatial and temporal variation in the collection of evidential samples, both control and experimental. Under experimental conditions, diatoms transfer in relatively high numbers following brief contact with aquatic sites, highlighting the potential for diatom analysis in a range of forensic contexts. Small scale spatial variability should be considered in the collection of a representative control sample for comparison. This investigation therefore concludes that multiple control and experimental clothing samples should be collected and analysed in order to account for such variability in the transfer of diatoms to recipient surfaces.

Initial empirical data have been provided in this study to support the use of diatom analysis as a valuable independent technique for the assessment of geoforensic trace evidence. This study provides an insight into the most effective methods for the collection of diatom particulates on a variety of substrates within criminal investigation. Finally, the findings contribute to the construction of an evidence base that will enable diatom evidence to be accurately interpreted and presented in a court of law.

Acknowledgements

The authors acknowledge that this research was funded by the Engineering and Physical Sciences Research Council of the UK through the Security Science Doctoral Research Training Centre (UCL SECRet) based at University College London (EP/G037264/1). The authors also wish to acknowledge the laboratory facilities in the UCL Department of Geography and the UCL Department of Earth Sciences. Thank you to Jim Davy and Janet Hope for their continued assistance and support.

References

- [1] R.M. Morgan, P.A. Bull, The philosophy, nature, and practice of forensic sediment analysis, *Prog. Phys. Geog.* 31 (2007) 43–58.
- [2] R.M. Morgan, P.E. Wiltshire, A. Parker, P.A. Bull, The role of forensic geoscience in wildlife crime detection, *Forensic Sci. Int.* 162 (2006) 152–162.
- [3] R.M. Morgan, J.C. French, L. O'Donnell, P.A. Bull, The reincorporation and redistribution of trace geoforensic particulates on clothing: an introductory study, *Sci. Justice* 50 (2010) 195–199.
- [4] A. Ruffell, J. McKinley, Forensic geoscience: applications of geology, geomorphology, and geophysics to criminal investigations, *Earth Sci. Rev.* 69 (2005) 235–247.
- [5] A.G. Browne, The use of forensic botany and geology in war crimes investigations in NE Bosnia, *Forensic Sci. Int.* 163 (2006) 204–210.
- [6] R.M. Morgan, M. Little, A. Gibson, L. Hicks, S. Dunkerley, P.A. Bull, The preservation of quartz grain surface textures following vehicle fire and their use in forensic enquiry, *Sci. Justice* 48 (2008) 133–140.
- [7] R.M. Morgan, J. Robertson, C. Lennard, K. Hubbard, P.A. Bull, Quartz grain surface textures of soils and sediments from Canberra, Australia: a forensic reconstruction tool, *Aust. J. Forensic Sci.* 42 (2010) 169–179.
- [8] R.M. Morgan, P.A. Bull, Sediment fingerprints: a forensic technique using quartz sand grains, *Sci. Justice* 46 (2006) 107–124.
- [9] A.J. Newell, R.M. Morgan, L.D. Griffin, P.A. Bull, J.R. Marshall, G. Graham, Automated texture recognition of quartz sand grains for forensic applications, *J. Forensic Sci.* 57 (2012) 1285–1289.
- [10] P.E. Wiltshire, Consideration of some taphonomic variables of relevance to forensic palynological investigation in the United Kingdom, *Forensic Sci. Int.* 163 (2006) 173–182.
- [11] M. Horrocks, K.A. Walsh, Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart, *J. Forensic Sci.* 44 (1999) 417–420.
- [12] D.C. Mildenhall, An unusual appearance of a common pollen type indicates the scene of the crime, *Forensic Sci. Int.* 163 (2006) 236–240.
- [13] R.M. Morgan, P.A. Bull, Data interpretation in forensic sediment and soil geochemistry, *Environ. Forensics* 7 (2006) 325–334.
- [14] B.G. Rawlins, M. Cave, Investigating multi-element soil geochemical signatures and their potential for use in forensic studies, *Special Publications 232, Geological Society, London*, 2004, pp. 197–206.
- [15] F.C. Quaak, I. Kuiper, Statistical data analysis of bacterial r-FLP profiles in forensic soil comparisons, *Forensic Sci. Int.* 210 (2011) 96–101.
- [16] J. Horswell, S.J. Cordiner, E.W. Maas, T.M. Martin, K.B. Sutherland, T.W. Speir, A. M. Osborn, Forensic comparison of soils by bacterial community DNA profiling, *J. Forensic Sci.* 47 (2002) 350–353.
- [17] D.L. Hawksworth, P.E. Wiltshire, Forensic mycology: the use of fungi in criminal investigations, *Forensic Sci. Int.* 206 (2011) 1–11.
- [18] L.A. Dawson, W. Towers, R.W. Mayes, J. Craig, R.K. Väisänen, E.C. Waterhouse, The use of plant hydrocarbon signatures in characterizing soil organic matter, *Special Publications 232, Geological Society, London*, 2004, pp. 269–276.
- [19] Á. Carvalho, H. Ribeiro, R. Mayes, A. Guedes, I. Abreu, F. Noronha, L. Dawson, Organic matter characterization of sediments in two river beaches from northern Portugal for forensic application, *Forensic Sci. Int.* 233 (2013) 403–415.
- [20] V. Jones, Diatom introduction, in: S. Elias (Ed.), *Encyclopaedia of Quaternary Science*, Elsevier, 2013, pp. 476–484.
- [21] F.E. Round, *The Ecology of Algae*, Cambridge University Press, Cambridge, 1984.
- [22] C.R. Hardy, J.R. Wallace, Algae in forensic investigations, in: D. Hall, J. Byrd (Eds.), *Forensic Botany: A practical guide*, Wiley, Oxford, 2012, pp. 145–174.
- [23] F.A. Lotter, H.J.B. Hofmann, A. Marchetto, Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. I, climate, *J. Paleolimnol.* 18 (1997) 395–420.
- [24] H. Öberg, E. Norström, M.M. Ryner, K. Holmgren, L.O. Westerberg, J. Risberg, A. Muzuka, Environmental variability in northern Tanzania from AD 1000 to 1800, as inferred from diatoms and pollen in Lake Duluti, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 374 (2013) 230–241.
- [25] A. Pouličková, P. Hájková, K. Kintrová, R. Bat'ková, M. Czudková, M. Hájek, Tracing decadal environmental change in ombrotrophic bogs using diatoms from herbarium collections and transfer functions, *Environ. Pollut.* 179 (2013) 201–209.
- [26] J. Hawryshyn, K.M. Rühland, R. Quinlan, J. Smol, Long-term water quality changes in a multiple-stressor system: a diatom-based paleolimnological study of Lake Simcoe (Ontario, Canada), *Can. J. Fish. Aquat. Sci.* 69 (2012) 24–40.
- [27] X. Tan, X. Xia, Q. Zhao, Q. Zhang, Temporal variations of benthic diatom community and its main influencing factors in a subtropical river, China, *Environ. Sci. Pollut. Res.* (2013) 1–11.
- [28] R.W. Battarbee, Palaeolimnological approaches to climate change, with special regard to the biological record, *Quat. Sci. Rev.* 19 (2000) 107–124.
- [29] G. Incze, Fremdkörper im blutkreislauf ertrunkener, *Zentralbl. Allg. Pathol. Anat.* 79 (1942) 176.
- [30] M.H. Piette, E.A. De Letter, Drowning: still a difficult autopsy diagnosis, *Forensic Sci. Int.* 163 (2006) 1–9.
- [31] J. Hürlimann, P. Feer, F. Elber, K. Niederberger, R. Dirnhofer, D. Wyler, Diatom detection in the diagnosis of death by drowning, *Int. J. Legal Med.* 114 (2000) 6–14.
- [32] T. Delabarde, C. Keyser, A. Tracqui, D. Charabidze, B. Ludes, The potential of forensic analysis on human bones found in riverine environment, *Forensic Sci. Int.* 228 (2013) e1–e5.
- [33] S. Krstic, A. Duma, B. Janevska, Z. Levkov, K. Nikolova, M. Noveska, Diatoms in forensic expertise of drowning—a Macedonian experience, *Forensic Sci. Int.* 127 (2002) 198–203.
- [34] D.A. Casamatta, R.G. Verb, Algal colonization of submerged carcasses in a mid-order woodland stream, *J. Forensic Sci.* 45 (2000) 1280–1285.
- [35] K.A. Zimmerman, J.R. Wallace, The potential to determine a postmortem submersion interval based on algal/diatom diversity on decomposing mammalian carcasses in Brackish Ponds in Delaware, *J. Forensic Sci.* 53 (2008) 935–941.
- [36] A.J. Peabody, Diatoms in forensic science, *J. Forensic Sci. Soc.* 17 (1977) 81–87.
- [37] N.G. Cameron, A. Peabody, Forensic science and diatoms, in: J.P. Stoermer, E.F. Smol (Eds.), *The Diatoms: Applications for the Environmental and Earth Science*, Cambridge University Press, Cambridge, 2010.
- [38] P.A. Siver, W.D. Lord, D.J. McCarthy, Forensic limnology: the use of freshwater algal community ecology to link suspects to an aquatic crime scene in southern New England, *J. Forensic Sci.* 39 (1994) 847–853.
- [39] *U.S. v. Hoepfner*, 206 Conn. 278 (1988).
- [40] S. Uitdehaag, A. Dragutinovic, I. Kuiper, Extraction of diatoms from (cotton) clothing for forensic comparisons, *Forensic Sci. Int.* 200 (2010) 112–116.
- [41] R.M. Morgan, J. Flynn, V. Sena, P.A. Bull, Experimental forensic studies of the preservation of pollen in vehicle fires, *Sci. Justice* 54 (2014) 141–145.
- [42] R.M. Morgan, G. Davies, F. Balestri, P.A. Bull, The recovery of pollen evidence from documents and its forensic implications, *Sci. Justice* 53 (2013) 375–384.
- [43] R.M. Morgan, E. Allen, T. King, P.A. Bull, The spatial and temporal distribution of pollen in a room: forensic implications, *Sci. Justice* 54 (2014) 49–56.
- [44] M. Horrocks, S.A. Coulson, K.A. Walsh, Forensic palynology: variation in the pollen content of soil on shoes and in shoeprints in soil, *J. Forensic Sci.* 44 (1999) 119–122.
- [45] A.G. Brown, A. Smith, O. Elmhurst, The combined use of pollen and soil analyses in a search and subsequent murder investigation, *J. Forensic Sci.* 47 (2002) 614–618.
- [46] K. Inman, N. Rudin, The origin of evidence, *Forensic Sci. Int.* 126 (2002) 11–16.
- [47] R.M. Morgan, J. Cohen, I. McGookin, J. Murly-Gotto, R. O'Connor, S. Murrens, P.A. Bull, The relevance of the evolution of experimental studies for the interpretation and evaluation of some trace physical evidence, *Sci. Justice* 49 (2009) 277–285.

- [48] P.A. Bull, R.M. Morgan, A. Sagovsky, G.J.A. Hughes, The transfer and persistence of trace particulates: experimental studies using clothing fabrics, *Sci. Justice* 46 (2006) 185–195.
- [49] S. Uitdehaag, I. Kuiper, A method for the combined extraction of pollen, diatoms, and phytoliths from forensic soil sample, Poster Presentation at the 3rd Annual Soil Forensics International Conference, Edinburgh, UK, 2007).
- [50] I. Renberg, A procedure for preparing large sets of diatom slides from sediment cores, *J. Paleolimnol.* 4 (1990) 87–90.
- [51] R.W. Battarbee, V.J. Jones, R.J. Flower, N.G. Cameron, H. Bennion, L. Carvalho, S. Juggins, Diatoms, in: J.P. Smol, H.J.B. Birks, W.M. Last (Eds.), *Tracking Environmental Change Using Lake Sediments Volume 3: Terrestrial, Algal, and Siliceous Indicators*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001, pp. 155–202.
- [52] S.A. Papadodima, S.A. Athanaselis, E. Skliros, C.A. Spiliopoulou, Forensic investigation of submersion deaths, *Int. J. Clin. Pract.* 64 (2010) 75–83.
- [53] P. Giannelli, Wrongful convictions and forensic science: the need to regulate crime labs, *North Carolina Law Rev.* 86 (2011) 163.
- [54] K. Krammer, H. Lange-Bertalot, Süßwasserflora von Mitteleuropa. Bacillariophyceae, Teil 1: Naviculaceae, Gustav Fischer, Stuttgart, 1986.
- [55] K. Krammer, H. Lange-Bertalot, Süßwasserflora von Mitteleuropa. Bacillariophyceae. 2. Teil: Epithemiaeae, Bacillariaceae, Surirellaceae, vol 2/2, Gustav Fischer, Stuttgart, 1988.
- [56] K. Krammer, H. Lange-Bertalot, Süßwasserflora von Mitteleuropa, Bacillariophyceae, 3 Teil: Centrales, Fragilariaceae, Eunotiaceae, Band 2/3, Gustav Fischer, Stuttgart, 1991.
- [57] K. Krammer, H. Lange-Bertalot, Bacillariophyceae. Teil 4. Achnantheaceae. Kritische Ergänzungen zu Navicula. Süßwasserflora von Mitteleuropa, Bd. 2, Gustav Fischer, Stuttgart, 1991.
- [58] S. Juggins (2010), C2 (Version 1.7.3): <http://www.staff.ncl.ac.uk/staff/stephen.juggins/software>.
- [59] B.J. Biggs, The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems, *Freshwater Biol.* 33 (1995) 419–438.
- [60] C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during simulated contacts and their persistence during wear: part II—fibre persistence, *J. Forensic Sci. Soc.* 15 (1975) 29–37.
- [61] C.A. Pounds, K.W. Smalldon, The transfer of fibres between clothing materials during simulated contacts and their persistence during wear: part I – fibre transference, *J. Forensic Sci. Soc.* 15 (1975) 17–27.
- [62] T. Hicks, R. Vanina, P. Margot, Transfer and persistence of glass fragments on garments, *Sci. Justice* 36 (1996) 101–107.
- [63] J. Dachs, I.J. McNaught, J. Robertson, The persistence of human scalp hair on clothing fabrics, *Forensic Sci. Int.* 138 (2003) 27–36.