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The transfer of diatoms from freshwater to footwear materials: An experimental study assessing transfer, persistence, and extraction methods for forensic reconstruction

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ABSTRACT

In recent years there has been growing interest in environmental forms of trace evidence, and ecological trace evidence collected from footwear has proved valuable within casework. Simultaneously, there has been growing awareness of the need for empirical experimentation to underpin forensic inferences. Diatoms are unicellular algae, and each cell (or ‘frustule’) consists of two valves which are made of silica, a robust material that favours their preservation both in sediments and within forensic scenarios. A series of experiments were carried out to investigate the transfer and persistence of diatoms upon common footwear materials, a recipient surface that has historically been overlooked by studies of persistence. The effectiveness of two novel extraction techniques (jet rinsing, and heating and agitation with distilled water) was compared to the established extraction technique of hydrogen peroxide digestion, for a suite of five common footwear materials: canvas, leather, and ‘suede’ (representing upper materials), and rubber and polyurethane (representing sole materials). It was observed that the novel extraction technique of heating and agitation with distilled water did not extract fewer diatom valves, or cause increased fragmentation of valves, when compared to peroxide digestion, suggesting that the method may be viable where potentially hazardous chemical reactions may be encountered with the peroxide digestion method.

Valves could be extracted from all five footwear materials after 3 min of immersion, and more valves were extracted from the rougher, woven upper materials than the smoother sole materials. Canvas yielded the most valves (a mean of 2511/cm²) and polyurethane the fewest (a mean of 15/cm²). The persistence of diatoms on the three upper materials was addressed with a preliminary pilot investigation, with ten intervals sampled between 0 and 168 h. Valves were seen to persist in detectable quantities after 168 h on all three upper materials. However, some samples produced slides with no valves, and the earliest time after which no diatom valves were found was 4 h after the transfer. Analysis of the particle size distributions over time, by image analysis, suggests that the retention of diatoms may be size-selective; after 168 h, no particles larger than 200 μm² could be found on the samples of canvas, and -95% of the particles on the samples of suede were less than or equal to 200 μm². A pilot investigation into the effects of immersion interval was carried out upon samples of canvas. Greater numbers of valves were extracted from the samples with longer immersion intervals, but even after 30 s, >500 valves could be recovered per cm², suggesting that footwear may be sampled for diatoms even if the contact with a water body may have been brief. These findings indicate that, if the variability within and between experimental runs can be addressed, there is significant potential for diatoms to be incorporated into the trace analysis of footwear and assist forensic reconstructions.

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1. Introduction

In recent years, practitioners, researchers, and regulators have identified the need for further empirical experimentation to provide an evidence base for forensic inferences [1–3]. This drive for research has both external motivation, as successive regulatory reports have identified large lacunae within the forensic literature [3–6], and also a legal
imperative, as experimental testing and peer-reviewed publication are amongst the prerequisites for scientific evidence to be considered admissible in court within the United Kingdom and USA [2,7].

The processes of transfer and persistence have been explicitly cited as a priority for research, with the United Kingdom’s Forensic Science Regulator’s Annual Report calling for “…structured studies into transfer and persistence of trace evidence and the significant factors affecting such transfer” [4]. While Locard’s exchange principle is often held as axiomatic [8–12], a nuanced understanding of the processes of transfer and persistence, underpinned by empirical research, is fundamental for the meaningful interpretation of trace evidence [1]. It is, therefore, necessary to undertake structured studies to establish under what conditions transfer occurs, in what quantities, and what kind of degradation can be expected in specific forensic scenarios [13–15].

Simultaneously, recent years have also seen a growing interest in environmental forms of trace evidence; the use of mineral grains [16–18], palynomorphs [19–21], bryophyte shoots [22,23], and algae [24,25] to exclude (or fail to exclude) contact between suspects, victims, objects, and crime scenes within forensic scenarios. One form of evidence that has proved particularly useful within aquatic environments is diatoms; unicellular algae that have traditionally found forensic application within the field of pathology [25–28]. Composed of two silica valves linked by a girdle element, the diatom frustule can be well-preserved within sediments and forensic contexts [29]. These algae are, at the generic level, widely distributed in aquatic environments, but at the level of species have narrow tolerances for environmental factors such as salinity, water temperature, and pH [25,29,30]. This means that many samples of water or damp sediment are likely to yield diatoms, and from the species composition of the diatom assemblage, it is possible to infer characteristics about the water body from which they originated [29,31]. It is because of this property that diatoms are employed widely within the environmental sciences, aiding environmental monitoring and palaeoenvironmental reconstruction [32–34]. This trait also means that diatom assemblages have been employed in forensic contexts to infer where a body might have drowned [26,35], if a corpse might have been moved from one body of water to another [36,37], and whether a suspect might have come into contact with a particular water body [24,25].

Previous studies have considered the transfer of diatom valves to clothing fabrics, and methods appropriate for their extraction [38,39]. As of yet, however, no published research has considered their transfer to footwear materials or their persistence. Accordingly, this research seeks to address this lacuna, undertaking a series of exploratory experiments on the transfer and persistence of diatoms upon common footwear materials, a recipient surface that, while valuable, has historically been under-represented in the literature on transfer and persistence [40,41].

Specifically, this research was designed to assess:

1. Whether diatoms transfer to common footwear materials after short periods of immersion in freshwater, and to what extent the initial levels of transfer are influenced by the recipient material type and duration of immersion;
2. Whether diatoms can be recovered from common footwear materials 168 h (7 days) after the transfer event, and to what extent the recipient material type appears to influence the timescales over which diatoms persist;
3. Whether diatom valves of different sizes appear to persist at different rates (i.e. whether retention is size-selective);

In order to address these questions, it is also necessary to consider how to extract diatoms safely and reliably from common footwear materials. There is, at present, no consensus on how best to extract diatoms for forensic purposes, neither for samples of fabric [38,39,42,43], or organic tissue [44–46]. No published research has specifically considered protocols for the extraction of diatoms from footwear. It was considered valuable to develop an alternative to the hydrogen peroxide method adapted from the environmental sciences [38,47] to ensure the ability to apply it to any footwear material – being a powerful oxidising agent, there is the potential for a combustible reaction when using hydrogen peroxide, especially when applying heat [48]. The ethanol rinsing method requires a shaker capable of generating turbulence [42,43], which was not available for this research. Accordingly, in this study, the hydrogen peroxide extraction method established by Scott et al. [38] (for the materials where this would not be hazardous) is compared with two novel techniques known to be safe with organic materials. Consequently, the fourth question addressed in this paper is:

4. Do any of the three extraction techniques extract significantly higher numbers of diatom valves, or cause a higher proportion of the diatom valves to fragment?

2. Materials and methods

2.1. Donated particles and recipient surfaces

Where experiments deal with the first principles of transfer and persistence, it is common to opt for highly-controlled simulated contact in order to create a uniform and reproducible distribution of the particles or fibres under investigation [13,49,50]. Accordingly, in this experiment, contact between footwear and a water body was simulated by immersing swatches of fabric in a ‘tank’ of diatom suspension. A suite of five fabrics was chosen: canvas, leather, and faux suede (100% polyester), representing uppers, and rubber and polyurethane representing soles. These materials were selected as demonstrative of both synthetic and natural uppers and soles, with variation in surface textures (Fig. 1).

To create a diatom suspension, water was collected from the Oxford canal, in the Upper Thames Valley (Fig. 2). This water contained approximately 150 diatom valves/cm², and the assemblage of diatoms was relatively diverse: predominantly Melosira varians, Fragilaria fasciculata, and Staurosirella pinnata, with less abundant numbers of Staurastrum leptosphaerum, Diatomite tenue, and Synedra ulna, in addition to species of Navicula, Gyrosigma, Stephanodiscus, Surirella, Nitzschia, Amphora, and Cyclotella. All of these genera are characteristic of British freshwater environments [51].

2.2. Experimental transfer methodology

Pristine (new) 4 cm² swatches of the five materials were immersed in the ‘tank’ of the collected canal water, and the tank was agitated to keep the algae and sediment in suspension. This water was not refreshed between experiments. For the persistence study, the swatches were immersed while attached to a new (unworn) pair of Wellington boots. These swatches (three of each material for each time interval) were attached to the boots with a staple-gun, so as to avoid introducing any adhesives, and to ensure that the area affected by the attachment was consistent for each swatch. The swatches were attached to the sides of the boots, approximately 2 cm above the sole. For the extraction and persistence studies, the swatches were immersed for 3 min. For the experiment in which the duration of immersion was an independent variable, groups of five canvas swatches were immersed for 30 s, 3 min, and 30 min. After immersion samples were allowed to dry, and stored in individual, airtight boxes, to prevent cross-sample contamination.

2.3. Persistence study methodology

The modified Wellington boots, which had been immersed in the tank of diatom solution for three minutes, were worn during waking hours over seven days while normal day-to-day activity was conducted (with approximately 15 min of activity per hour). Three swatches of the three upper materials (canvas, leather, and ‘suede’) were removed at ten time intervals: at 0.0, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 36.0, and...
168.0 h after immersion. Given the preliminary nature of this study, the effect of the position of the sample on the shoe (i.e. whether it was closer to the toe or heel of the shoe) was not investigated. It is acknowledged that this is a variable that should be addressed in future experimental work.

2.4. Extraction methods

Swatches of the five upper and sole materials that had been immersed for 3 min were subjected to three different extraction treatments. Method 1 (Jet Rinsing) consisted of rinsing the fabric sample with a pressurised jet of 100 cm³ of distilled water, into a beaker. This water was centrifuged at 1200 rpm for 4 min, so that the diatom valves collected in a pellet at the bottom of the centrifuge tube. The supernatant was suctioned off to leave the pellet and 5 cm³ of water, which were then homogenised, so that the diatoms were suspended within those 5 cm³ of solution.

Hydrogen peroxide digestion was the second method employed, as outlined in [38]. In pristine centrifuge tubes, the fabric samples were heated with H₂O₂ (volume: 30 cm³, concentration: 30%) at 70 °C for 3 h. The fabric sample was then removed, rinsed with distilled water, and HCl (19%) was added to the solution into order to neutralise the peroxide, and eliminate carbonates. After three cycles of centrifuging and rinsing, the last supernatant was suctioned off to leave the diatom pellet in 5 cm³ of solution, which was then homogenised. For the samples where the addition of hydrogen peroxide would have been potentially hazardous (leather, rubber, and polyurethane), distilled water was substituted, and the samples were submitted to the same regime of heating, centrifuging, and rinsing (without the addition of hydrochloric acid).

The third extraction method involved the application of heat and agitation. Each fabric sample was placed in a pristine centrifuge tube with 30 cm³ of distilled water, and heated in a Clifton NE-5 shaking water bath at 70 °C and 225 strokes/min for 3 h. The fabric sample was then removed, rinsed with distilled water, and the water sample was centrifuged at 1200 rpm for 4 min. The supernatant was suctioned off, to leave the pellet of diatoms and 5 cm³ of sample solution, which were then homogenised, without hydrogen peroxide cleaning.

Fig. 1. Images of the five recipient materials at 1× and 50× magnification (micrographs taken on a Jeol JSM-6480LV Scanning Electron Microscope, with an acceleration voltage of 7 kV).

Fig. 2. Location of the water sampling site (Oxford, UK).
Following the results of the pilot extraction experiment, this third method, of heating and agitating the samples, was employed to extract the samples in the immersion interval and persistence studies. For all combinations of variables, blank samples (with reagents but no fabric) and control samples (with fabric that had not been exposed to diatoms) were prepared.

2.5. Slide preparation

After extraction, all sample solutions were treated with the same slide-making process. An Eppendorf calibrated micropipette was used to transfer 0.5 cm³ of each 5 cm³ solution onto a coverslip. A second dilution of each suspension, at half the concentration, was also prepared in this manner. In order to prevent contamination between samples, a fresh tip was attached to the calibrated micropipette for each sample. After evaporation, slides were produced following the standard procedure for mounting diatoms in environmental science; Naphrax™ (with a refractive index of 1.73) was used as the mounting medium, and a hotplate was used to drive off the toluene [30,52].

2.6. Slide analysis

Slides were examined under phase contrast microscopy. Continuous transects were performed over 50% of the surface area of the slide. Information about the morphology of the valve, and whether it was fragmented or whole, was recorded. Where whole cells, or chains of cells, were encountered, the count was standardised to valves (i.e. one cell = two valves). In order to convert these data into units that could be meaningfully compared with the findings of other studies, counts were multiplied to express the number of valves extracted per cm² of material, following [38]. In total, 153 sample slides were analysed, in addition to blanks and controls.

2.7. Measuring particle size distribution

The persistence study slides were again examined under phase contrast microscopy, and continuous transects were scanned over the previously unexamined half of the slide. Every time a valve, cell, or chain of cells was encountered a micrograph was taken, using a Leica ICC50W HD digital camera. Adobe Photoshop was used to collate the micrographs for each half-slide into a single image, representing all of the diatom valves seen in all fields of view on that half-slide. A threshold filter was applied, to enhance contrast between the silica frustules of the diatoms and the background of the slide. Any holes in the outlines of the frustules were manually corrected with the opacity of the thresholded layer lowered, to confirm that the reconstruction was an accurate representation of the original micrograph. These images were then imported into ImageJ, and after calibrating for scale and using a colour threshold to select the darker particles (not the background) as the region of interest, the ImageJ “analyse particles” function was used. Particles smaller than 3 μm² were excluded from the analysis, in order to avoid mistaking any noise introduced by the threshold filter as relevant particles. Performing this analysis resulted in a table of particle areas, and an image displaying the contours identified by ImageJ, with each particle identified by a number corresponding to the table row. For each image, the original image of collated micrographs (input) was checked against this second image (output), to make sure that no discrepancies existed.

2.8. Statistical analysis

This paper presents the results of preliminary pilot investigations. Since the sample sizes are small, and we do not have a reliable measurement of the population variance, inferential statistical analysis is not presented. Instead, following the presentation of count data in both [38] and [39], the mean and standard deviation are provided.

3. Results

3.1. Extraction

3.1.1. The numbers of valves extracted

For the four materials of ‘suede’, leather, rubber, and polyurethane, the three treatments appeared to extract similar numbers of valves (Fig. 3). For the canvas samples, Method 1 (Jet Rinsing) appeared to recover fewer valves than Method 3 (heating at 70 °C and shaking at 225 strokes/min).

3.1.2. The fragmentation of valves

In the diatom suspension 4 ± 1% of the valves were fragmented. For the three materials of canvas, suede, and leather, there does not appear to be a large difference in the mean percentage of valves fragmented by the three extraction techniques (Fig. 4), with aggregated means of 5 ± 2%, 5 ± 2%, and 6 ± 6% respectively. For rubber and polyurethane, all three methods seemed to produce slides with a higher and more variable proportion of fragmented valves.

3.2. Transfer

3.2.1. Variation between materials

Diatoms were extracted from all five materials after 3 min of immersion. Larger numbers of valves were extracted from the two woven fabrics than from the three smoother materials (Fig. 3); if one aggregates the results of the three extraction methods, canvas yielded a mean of 2511 ± 1225 valves/cm² and suede 1780 ± 509/cm², while the means for leather (193 ± 259/cm²), rubber (121 ± 70/cm²), and polyurethane (15 ± 14/cm²) are much lower.

3.2.2. The effect of immersion interval

The effect of immersion interval was only tested upon canvas, to see if this variable was worth further exploration. Valves were recovered from all samples. After only 30 s, 614 ± 187 valves were extracted per cm² of canvas, and increasing numbers of diatoms were extracted...
from the samples with longer submersion intervals; 1249 ± 415/cm² after 3 min, and 2495 ± 814/cm² after 30 min (Fig. 5).

3.3. Persistence

3.3.1. Absolute numbers of valves

Valves were recovered from samples of all three upper materials at all time intervals (Fig. 6), however, some samples produced slides which contained no valves (leather at 4 and 24, and 168 h) (Fig. 6C). For all three materials, the data generated by these experiments are noisy, with some later time intervals yielding higher numbers of valves than earlier time intervals (for example, see between 12 and 24 h for canvas, or 8 and 12 h for suede), and with the three replicates showing considerable variation.

3.3.2. Relative numbers of valves

Presenting the data as relative (%), rather than absolute values, none of the three materials exhibit an ‘exemplar’ decay curve, of the sort demonstrated by Pounds and Smalldon [53]. All three materials
produced data points which exceed the initial reading at 0 h, and all three appear to show increases as well as decreases over time (Fig. 7). In the 168 h covered by this study, none of the materials declined to 0% of the initial transfer, which is consistent with the previously published ‘exemplar’ decay curve results.

3.3.3. Fragmentation over time
Considering the proportion of valves in each sample that were fragmented, no clear trend was seen over time (Fig. 8). While the canvas samples did exhibit a weak upwards trend, with a smaller proportion of whole valves recovered as time elapsed, this trend was not strong, and not seen in the data for suede or leather.

3.4. Particle size distributions over time
Analysis was not conducted on the leather slides, since the small numbers of diatoms precluded meaningful plots of particle size distribution. For canvas (Fig. 10) and suede (Fig. 11), the data suggest that larger particles were lost more rapidly than smaller particles. For canvas, the distribution generated immediately after immersion (0 h) closely resembles the distribution found within the tank solution (Fig. 9), containing diatoms and chains of diatoms of many sizes, including 700–800 μm², 800–900 μm², 900–1000 μm², 1200–1300 μm² and 1300–1400 μm² (Fig. 10). After 1 h, the maximum particle size observed is 600–700 μm², implying that the larger diatoms and chains have been lost from the shoe (Fig. 10). This trend continues; the largest particle size observed after 4 and 24 h is between 500 and 600 μm², and no particles larger than 100–200 μm² are present in the sample from 168 h after contact. A similar trend is observed for the suede samples; particles of between 1300 and 1400 μm² are observed at 0 h, and in no subsequent samples. After 168 h, over 95% of the particles recovered are up to 200 μm², and <1% (0.78%) were of the 400–500 (0.39%) and 700–800 μm² (0.39%) size categories. In summary, for both materials very long chains of diatoms (between 1200 and 1400 μm²) were observed only in the samples at 0 h, and after 168 h of wear, the samples for canvas exclusively (100%), and for suede predominantly (in excess of 95%), consisted of particles of less than or equal to 200 μm².

3.5. Control and blank samples
No diatom valves were observed on any of the slides prepared from blank and control samples.

4. Discussion

4.1. Extraction
No large differences were observed between the mean numbers of valves extracted unit area of fabric, nor between the mean proportions of valves fragmented, when comparing the hydrogen peroxide digestion method [38,47], to the method of heating and agitating the samples in distilled water.

While it is acknowledged this is a modest dataset (with only three replicates for each combination of variables), this suggests that the technique of heating and agitation might be a viable alternative to peroxide digestion where samples could react hazardous with the reagent. This avoidance of potentially hazardous reactions is particularly advantageous in the forensic context, where an examiner may not necessarily know the material composition of an exhibit. Accordingly, further research to compare the method of heating and agitation with ethanol rinsing would be desirable, since it has been established that different forensic methodologies can exhibit varying efficacy upon different substrates.

Regarding the variation in the proportion of fragmentation between materials, it is likely that this is an artefact of the population size, rather than a genuine difference; where fewer valves were recovered, a small number of fragmented valves would appear to form a larger proportion of the population.

4.2. Transfer
Valves were extracted from all materials used in this study, after even brief periods of immersion (30 s immersion resulted in a minimum of 425 and a mean of 614 valves being recovered per cm² of canvas). Both the duration of immersion and the recipient surface material appeared to be important factors for the levels of transfer; after 3 min
of immersion, a mean of 2511 valves/cm² could be recovered from samples of canvas, 1780 valves/cm² could be recovered from suede, 193 valves/cm² could be recovered from leather, 121 valves/cm² could be recovered from rubber, and 15 valves/cm² could be extracted from the samples of polyurethane.

These relatively high amounts of recovered diatoms suggest that in contexts where diatoms may be useful trace evidence, it is worth sampling footwear even if the duration of contact with the water body may have been short. It also implies that where shoes are made of several different materials (for example training shoes, which often have panels of breathable mesh, rubber soles, and other fabric accents), it is likely to be most fruitful to sample woven fabrics on the uppers for diatoms, in preference to smoother rubbers or plastics on the soles.

These findings accord with existing published studies on the transfer of trace particulates, which suggest that rougher surfaces tend to entrain larger numbers of particulates than smoother materials [54–56], a trend also seen in experiments involving hairs and fibres [13, 49, 57–60].

### 4.3. Persistence

It was possible to recover valves from samples of canvas, suede, and leather after 168 h of wear, and at all intervals tested in this study. This in itself is an important finding, as this is the first time that a structured study has been undertaken upon the persistence of diatom valves on footwear materials. This study implies that the analysis of diatoms from footwear in a forensic context may still be viable if a week has elapsed between the contact event and the evidence being recovered. The fact that particulates in the size range of 20–200 µm were found to persist in detectable amounts on common footwear materials for 168 h is in accordance with the literature, with similar findings in the study by Bull et al. concerning the persistence of palynomorphs on common clothing materials [56]. The data also, however, suggest the need for caution when interpreting evidence; some samples yielded slides containing no valves, despite exposure to the diatom population. It is possible that this was caused by a non-homogenous distribution of the algae in the water sample.

These data exhibit a degree of variability which may be a product of the destructive sampling procedure. As the process of quantification involved the removal of samples, it was not physically possible to monitor the concentration of diatoms on the same region of fabric at the same location over time. Instead, different areas of fabric removed at different times were used as a proxy for repeat measurements of the same location of the shoe. Given the variation seen both between and within time interval samples, it is possible that the transfer of valves to these different locations may not have been uniform, even under these very controlled experimental conditions, or that diatoms were lost from different locations on the shoe (e.g. closer to the toe versus closer to the heel) at varying rates – and failing to control for this variable might account for the apparent increases in diatoms found in later time intervals. It is also possible that the swatches could have been contaminated by dust (including diatoms) and water spray, or that variation in the levels of activity over the 168 h could have affected the reproducibility of the data.

Similar problems with destructive sampling methodologies generating noisy data have been encountered in studies employing gunshot residue (GSR), where sampling by swabbing also precludes repeated sampling of the same location [61]. In contrast, it appears that where repeated non-destructive (e.g. photographic) sampling is undertaken, much smoother decay curves with no anomalies are seen [62].
Fig. 10. Particle size distributions of the diatom samples extracted from canvas, over time.
Accordingly, further research could consider a non-destructive sampling methodology to interrogate particulate trace evidence dynamics upon footwear. Utilising a proxy for particulate evidence, such as UV powder which can be quantified using photography, may be a viable option, as has been seen in recent explorations of the trace evidence dynamics of fungal spores [62] and pollen [63].

4.4. Particle size distributions over time

For both canvas and suede, it appeared that larger particles were lost more rapidly than smaller particles. For both materials, very long chains of diatoms (between 1200 and 1400 μm²) were observed only in the samples at 0 h. After 168 h of wear, the samples for canvas exclusively (100%), and for suede predominantly (in excess of 95%), consisted of particles of less than or equal to 200 μm². These data suggest that the size-selective retention observed in studies of glass [54], fibres [58], and soils [64] might also apply to the retention of diatoms. Further research could be conducted, considering a broader suite of materials, and greater sample sizes. Further research should consider whether this size-sorting has an effect upon the species composition of samples — which would be forensically relevant information for the comparison between exhibit and exemplar samples.

5. Conclusion

This aim of this study was to identify the transfer of diatom valves to common footwear materials, their persistence on these fabrics, and methods of extracting the valves from these substrates for analysis. It was found that diatoms could be extracted from swatches of canvas, 'suede', leather, rubber, and polyurethane after even short periods of immersion, and that valves could be recovered from all three upper materials after 168 h of wear. (However, some samples produced slides...
Fig. 11. Particle size distributions of the diatom samples extracted from suede, over time
containing no diatoms, and the earliest time after which this was observed was 4 h.) A novel extraction technique, involving the heating and agitation of the sample with distilled water, was utilised and led to the extraction of a similar number of valves in comparison to the hydrogen peroxide method adapted from the environmental sciences, without causing a higher level of fragmentation (which might preclude species identification).

The implications of the transfer and persistence experiments are fourfold;

1. Footwear is a recipient surface that can be sampled for diatoms in forensic scenarios;
2. Sampling may be viable even when contact with a water body may have been brief;
3. Where shoes are composed of a mixture of fabrics, it may be more fruitful to sample coarser, woven materials;
4. The analysis of diatoms recovered from footwear may be viable even if 168 h have elapsed between the contact and the evidence being recovered. It appears to be likely that the <200 μm² fraction will be retained after such time periods.

Together, these findings confirm that footwear can represent a useful repository of diatoms in casework scenarios for both forensic reconstruction and the generation of forensic intelligence and evidence.

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N.B. 'N' here refers to the number of valves, cells, and chains of cells analysed.

Fig. 11 (continued).
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