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Short Communication

# Macroscopic assessment of environmental trace evidence dynamics in forensic settings

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ABSTRACT

Environmental trace evidence offers useful circumstantial intelligence to link persons and scenes of forensic interest. An increasing empirical research base is dedicated towards understanding the transfer and persistence dynamics of environmental indicators including pollen, soils, and diatoms, within a diverse range of experimental frameworks. This paper presents two discrete studies exploring transfer and persistence of soils and sediments on footwear and diatomaceous earth adhered to clothing in forensically pertinent scenarios. Variables including sediment type, foot position, clothing type, and body positioning were also explored throughout. Both experiments incorporated a field-based methodology during the sampling effort. Photographs were collected of an initial transfer sample and of a retained assemblage following hours, days, and up to one-week of wear, facilitating macroscopic assessment of trace evidence dynamics. All images were processed using accessible, open-source software before spatial analysis of evidence distribution within and temporal assessment (% retention) upon each evidential surface. The results highlighted consistent loss of transferred sediment from footwear with significantly greater retention of loamy clay soil than dune sand which was absent beyond 24 h of wear. Loss was not influenced by wearer gait but was more rapid from those areas of the shoe sole in direct contact with the ground. Diatomaceous earth was retrieved from all three clothing types tested after one week – significant losses of material occurred before 48 h with a consistent assemblage identified beyond this. Denim was significantly more effective than acrylic and fleece for diatomaceous earth retention and significantly more material was lost from clothing worn on the lower body. These findings highlight the value of using visual environmental markers and a macroscopic analytical approach during the investigation of environmental trace dynamics. The methodology offers a novel, non-destructive assessment of soil and diatom transfer and persistence, complementing more extensive laboratory-based examinations to ensure the development of a well-rounded research base within the forensic sciences.

## 1. Introduction

Environmental trace evidence analysis involves the study of geological and ecological traces from scenes and persons of forensic interest when reconstructing crime events [1]. Circumstantial indicators include soils and sediments, pollen, diatoms (algae), and microbes [2–5]; techniques including microscopy, chromatography (e.g., HPLC, GC–MS), and DNA metabarcoding often facilitate forensic identifications, sample discriminations, and exclusionary interpretations [1,3,4,6].

Empirical studies of environmental trace indicators - particularly in relation to their transfer, persistence, collection, and analysis – have increased in recent years [2,7–9] alongside calls for research and development within the forensic sciences [10]. For example, research has identified that fabric type significantly influences the extent of pollen, diatom, and dry soil transfer to clothing [8,9,11]; the distribution of soils transferred to footwear varies based on sole surface structure [2]; and cellular characteristics (e.g., pollen shape, diatom size) enable or limit the transferability of individual particulates to evidential surfaces [7,8]. Furthermore, longitudinal studies have identified the persistence of pollen [12] and freshwater diatoms [13,14] on clothing over one month and of diatoms on footwear [7] following one week of wear, and the retention of soil from prior locations on footwear despite subsequent environmental exposures [2,15]. Structured research studies

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such as these form important contributions to the empirical evidence bases required to support the collection, analysis, and interpretation of environmental traces in forensic casework [16].

Resource-intensive protocols are frequently utilized for the scrutiny of environmental traces and subsequent, quantitative, assessments of transfer and persistence dynamics. For example, previous studies have documented the use of electron (e.g., SEM) and light microscopy (e.g., phase contrast), with various stages of chemical preparation, to visualize the microscopic size and structures of soil quartz grain surface textures, pollen, and diatoms [17–19]. Although these methods offer a detailed and extensive insight into the diagnostic features of environmental traces, their application within forensic research or casework may not always be feasible or recommended if transferred evidence is not initially (visually) recognized [20]. Macroscopic observations of different substrates and different evidence types may therefore offer a valuable and cost-effective preliminary approach to explore different activity levels and subsequent impacts for environmental trace dynamics.

Previous research has primarily considered the transfer and persistence of trace evidence indicators within laboratory-controlled conditions [4,8,12], and/or using visual markers as identifiable proxies for particulate evidence [12,21–24]. Though these approaches are useful, the development of empirical studies which attempt to replicate the circumstances frequently encountered in forensic casework also generate valuable data to support the empirical evidence bases upon which robust forensic interpretations rely [16]. Subsequently, the experiments designed, presented, and discussed in this paper sought to replicate forensic settings as much as possible via field-based observations and the use of accessible technologies and software to facilitate analysis. This highlights the potential to develop novel, non-laboratory-based, widely applicable approaches for the empirical study of trace evidence dynamics within environmental forensic science.

This paper presents the findings from two discrete studies which sought to macroscopically assess the transfer and persistence of soils and sediments on footwear (experiment 1) and diatomaceous earth on clothing (experiment 2) following hours, days, and up to one week of wear. Both studies aimed to explore variation in the initial transfer and subsequent persistence of environmental traces through spatial and temporal assessment of total surface cover (%). The impact of sediment type (loamy clay soil v dune sand) and foot position (left v right) were considered as additional variables in experiment one, whilst the impact of clothing type (cotton, denim, acrylic) and body positioning (upper v lower) was incorporated within the second experiment.

## 2. Experiment 1: Sediment persistence on footwear

Soils and sediments are arguably the most frequently studied form of environmental trace evidence [25]. Previous research has demonstrated the development of multiple physical, chemical, and biological techniques to facilitate forensic comparisons and exclusions using different markers within the soil matrix [2,4–6,26]. For example, palynology [27] and quartz grain surface texture analysis [17] via SEM have previously been used to explore the transfer and persistence dynamics of soils transferred to footwear and vehicle surfaces from proposed crime scene environments. Furthermore, macroscopic analyses of soil transfer to and distribution upon the surface of different clothing items using image processing software to support visual observations, have also recently been reported within the forensic research base [9,28–29].

This study aimed to assess the distribution and persistence of soils and sediments (via total % surface cover) initially transferred to footwear from different environments following 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h (one week) of wear. The impact of sediment type was considered by comparing loamy clay soil and beach dune sand retention and both the left and right foot were analysed to determine whether identified wearer trends were consistent. Furthermore, a secondary aim sought to explore and validate the relationship between qualitative observations of soil persistence (via % cover) and the true mass (g) of a retained assemblage on footwear.

### 2.1. Methods

Ethical approval was not required in accordance with LJMU Faculty of Science guidance regarding observational work in the field. Consent was provided for data collection and sharing.

#### 2.1.1. Field protocol

Two sites were chosen that exhibited different underlying geologies and sediment types within the UK [30]. Initially, the persistence of loamy clay soil was tested following the transfer of material in Sefton Park (Liverpool, UK) (National Grid Ref: SJ375875), before subsequent analysis of dune sand retention following transfer from Pakefield Beach (Suffolk, UK) (National Grid Ref: TM539903) (Fig. 1).

At each location, a pair of walking boots (Peter Storm™, size 8) was initially worn and repeatedly walked across a 10 m transect for 30 min to initiate sediment transfer. A photograph of each shoe sole was immediately taken to document this initial transfer (0 h) assemblage using an iPhone XR© 12-megapixel camera (Fig. 2).

The shoes were worn, and walked, outdoors for up to 8 h per day during November and December 2020, reflecting the design of previous experimental protocols [7,12,13]. The distance travelled was approximately 4 miles for each day of the experiment. To limit the introduction of soil from additional, successive, locations during wear [2,15], the footwear was exclusively exposed to smooth tarmac concrete surfaces within an urban environment. The same individual (170 cm height, 63 kg weight) wore the shoes throughout the experiment to maintain consistency. Average step length was 63–74 cm, walking asymmetry was 1.8%, and walking speed ranged from 1.1 to 4.8 mph (Apple Watch data). The experiment continued regardless of weather conditions which included seasonal rain and wind. As in regular daily activity, surface irregularities (e.g., puddles) were avoided.

#### 2.1.2. Data collection

Photographs of the individual shoe soles documenting any remaining adhered sediment were subsequently taken 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h post-transfer (total n = 288). All photographs were taken at a $90^0$ angle with the shoe secured in place to ensure consistency. The experiment was repeated three times over three separate weeks at each site with the same wearer and the same pair of boots which were thoroughly cleaned between sample runs [2].

Each photograph was individually processed using Adobe Photoshop CC 2021. Using the full resolution image, a 2x2mm grid overlay was applied, accounting for the entire surface of each shoe sole (Fig. 2). If any sediment was present within each individual grid square, a positive result was recorded in red. If sediment was absent, a negative result was documented in black (Fig. 2). This ratio was then converted into a total percentage (%) cover. Mean values were subsequently calculated from subsample replicates (n = 3).

#### 2.1.3. Statistical analysis

Three-way repeated measures ANOVA was conducted to analyse the combined main effects of sediment type (soil; sand), footwear position (left; right), and persistence interval (0–168 h), and any interactions present on % surface cover over time. The data met the assumptions for the statistical analysis; sphericity was analysed using the Mauchly test [47]. Effect size was reported as partial Eta-squared ($\eta 2$) and was used to indicate the variance accounted for by each factor. Significant effects were further analysed using pairwise comparisons with Bonferroni correction applied for multiple comparisons. Analysis was performed in SPSS v. 28 using a 95% confidence interval.

#### 2.1.4. Comparison of percentage cover to true mass

To provide an initial insight into the mass (g) associated with

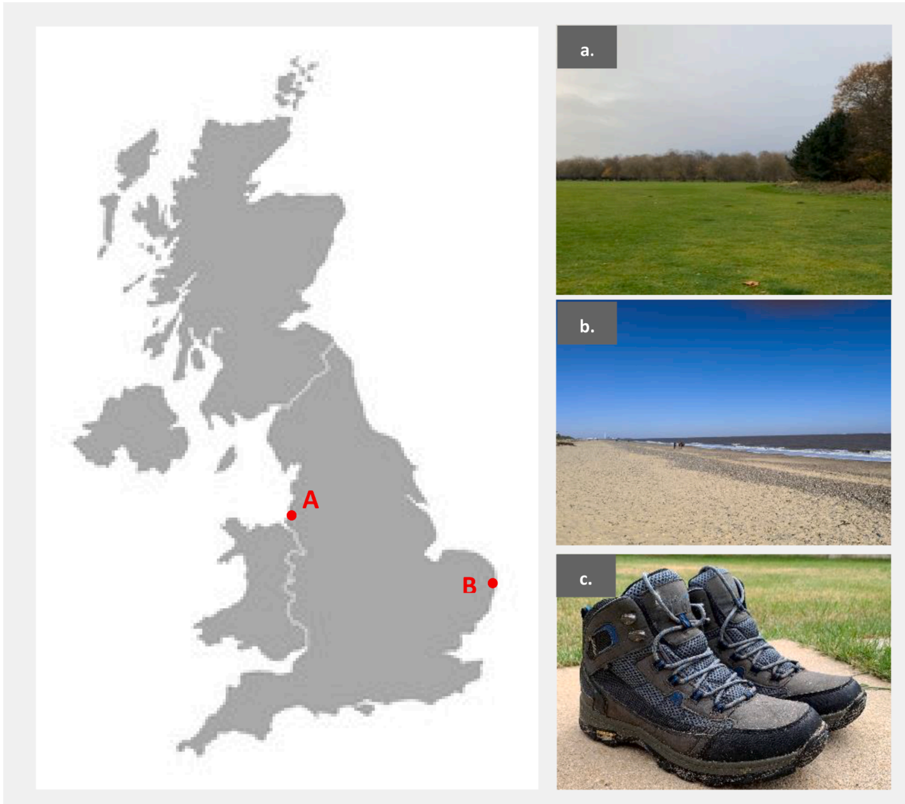



**Fig. 1.** Map and images of the two sites visited to initiate sediment transfer in Experiment 1 - Sefton Park in Merseyside (a) and Pakefield Beach in Suffolk (b). The same pair of shoes (Peter Storm™) were used throughout the study (c).

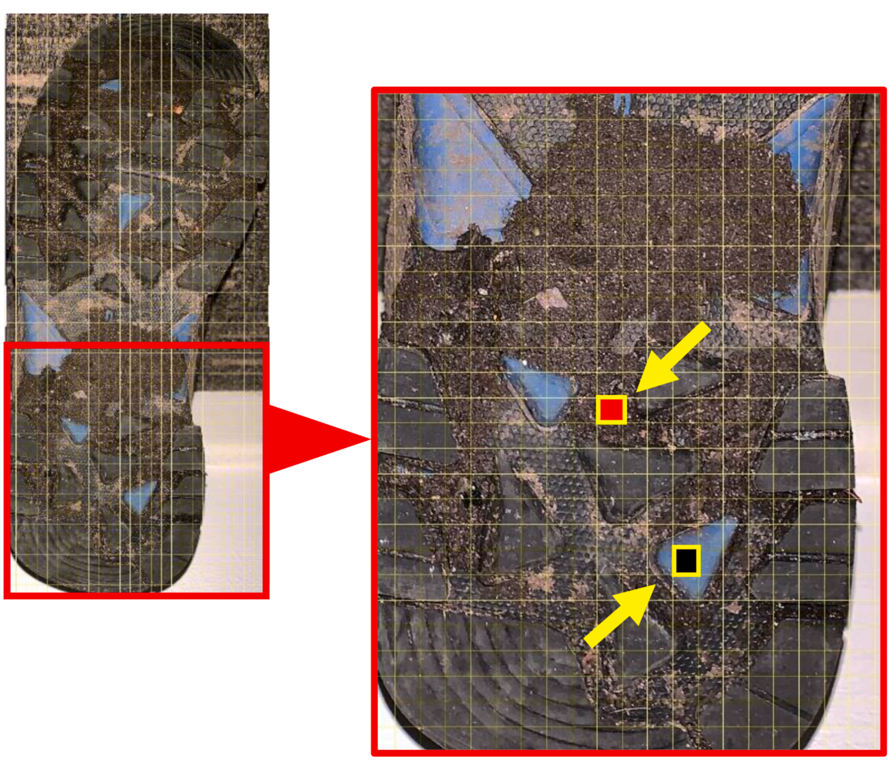


**Fig. 2.** Example of the 2x2mm grid overlay applied for the analysis of sediment percentage cover (%) on footwear using Adobe Photoshop CC in Experiment 1. The presence of sediment in each individual square was recorded as a positive result in red; the absence of sediment was marked in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

macroscopic analysis of % cover, a brief validation study was conducted. The same pair of shoes were exposed to the same environments and sediment types. Transfer was initiated using the same protocol until the footwear sole was loaded with different levels of surface coverage – approximately, low (<10%), low-medium (c. 25%), medium (c. 50%), medium–high (c. 75%) and high (>90%) cover. Actual percentage coverage was determined using the same image processing protocol as described. Each transferred sediment assemblage was subsequently removed using a hard bristle brush, collected, and weighed (g) using a field balance. Three replicates were prepared for each group and mean values were calculated.

### 2.2. Results

Initial assessment of spatial trends highlighted that loamy clay soil was more effectively transferred and retained on footwear over time (Table 1). Although sand was absent beyond 24 h, soil continued to be observed beyond one week (168 h) of wear. The initial transfer (0 h) of loamy clay soil was more uniformly distributed across the shoe sole, although persistent sediment was more localized to recessed areas of the middle and upper sole over time (168 h).

Subsequent analysis of mean percentage cover (%) identified these differences more clearly (Fig. 3). The initial loss of dune sand was rapid with only 59% (1 h), 13% (2 h), and 9% (4 h) of the initial transfer assemblage retained over time. In comparison, the proportion of loamy clay soil retained on the shoe sole was greater than 18% (168 h) throughout the experiment (Fig. 3a). No dune sand was identified beyond 24 h; at the corresponding persistence interval, 53.2% of the transferred soil sample remained and was visually identified. Persistence dynamics of both sediment types were consistent between the left and right foot for the duration of the experiment (Fig. 3b, c).

Three-way repeated measures ANOVA found no statistically significant three-way interaction between sediment type, footwear position, and persistence interval on % surface cover, $F(11, 22) = 1.632$, $p = .158$. Bonferroni adjustment was applied for the two-way interactions and simple main effects. A statistically significant two-way interaction was identified between sediment type and persistence interval, $F(11, 55) = 9.047$, $p < .0001$, with a large effect ($\eta 2 = .644$). The properties of the transferred sediment significantly influenced percentage retention within each persistence interval. Simple main effects identified that significantly more soil persisted over time in comparison to sand ($p = .004$, $\eta 2 = .828$) (Fig. 3a). No statistically significant two-way interaction was identified between the left and right foot and persistence interval, $F(11, 55) = 1.976$, $p = .05$. The loss of transferred material was consistent between both shoes ($p = .80$) (Fig. 3b, c). No statistically significant two-way interaction was identified between sediment type and footwear position, $F(1, 2) = .289$, $p = .645$. Sediment loss was not influenced by wearer gait over the course of the experiment.

Persistence interval had a large, significant simple main effect, $p < .0001$, $\eta 2 = .804$. Pairwise comparisons identified that significantly less sediment was present beyond 48 h of wear ($p < .05$) than in the earlier persistence intervals (0–24 h). The percentage of retained material was not significantly different between consecutive persistence intervals after 24 h – e.g., 24 h-48 h ($p = .70$ [± 6.27%]) and 48–72 h ($p = 1$ [± 1.71%]).

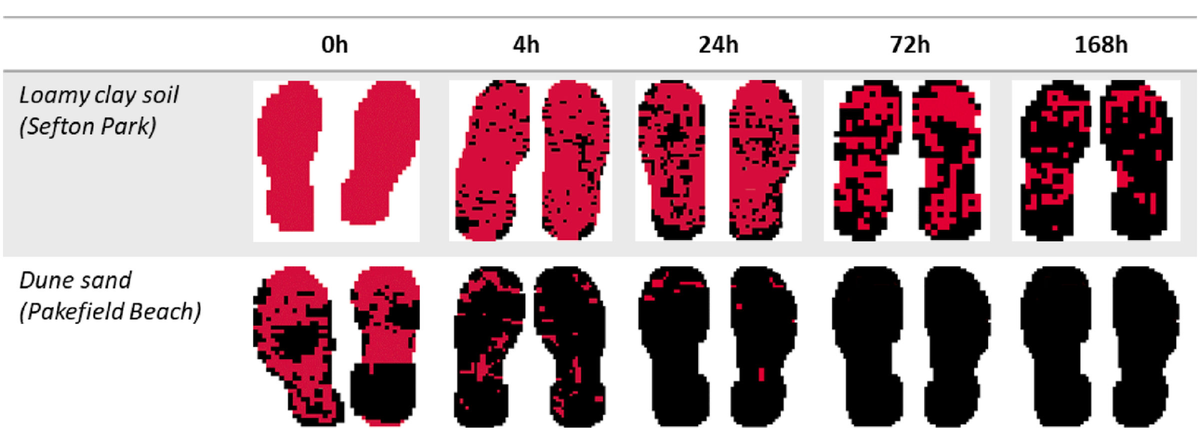
The true mass of a transferred assemblage in relation to % surface cover was initially explored (Table 2). As surface cover increased for both loamy clay soil and dune sand, the mass of a recovered sample also increased. For example, loamy clay soil yielded 8.7 g (54%), 17.4 g (69%), and 22.6 g (91%) of sediment as macroscopic increases in % cover were identified. The mass of dune sand retrieved was lower across all groups, with only 1.3 g of sediment identified in the low coverage samples (Table 2).

### 2.3. Discussion

This study, to our knowledge, is the first to explore sediment transfer and persistence on footwear using macroscopic analyses of spatial distribution and percentage surface cover over time. The results highlight a consistent loss of adhered material, with significantly less sediment retained beyond 24 h (sand) and 48 h (soil) of wear. Loamy clay soil was more effectively retained than dune sand, as demonstrated both in terms of percentage cover and sample mass (Fig. 3, Table 2). Transfer and persistence dynamics were consistent between the left and right shoe sole suggesting that an individual's gait does not impact on sediment loss from footwear over time.

Sediment persistence dynamics demonstrate broadly similar trends to those previously identified within fibre [31], diatom [7], blood [32], soil and pollen [12,33] research using footwear as a recipient surface. A two-stage model of decay was identified – first, a rapid, statistically significant loss, followed by consistently slower decay over time (Fig. 3a) [34]. Retention of 18–20% of the transferred loamy clay soil assemblage following one week of wear (Fig. 3c), corresponds with previous environmental forensic research where a 'significant amount' of sediment was retained on shoe soles after 450 m of walking [33], and where transferred diatoms were recovered and identified from footwear following a week of wear [7]. In contrast, no dune sand was identified beyond 24 h of wear (Fig. 3b), reflecting similar losses of an entire transferred fibre assemblage following five minutes of wear on concrete, grass, and carpeted surfaces [31]. Despite an initial sedimentary transfer taking place, the extent of trace retention on footwear over time is dependent on an array of factors including recipient surface properties,

**Table 1**
Initial spatial analysis of loamy clay soil and dune sand transfer (0 h) and retention at 4 h, 24 h, 72 h, and 168 h on footwear in Experiment 1. Areas marked in red indicate the presence of sediment whilst those areas in black indicate the absence of adhered material.



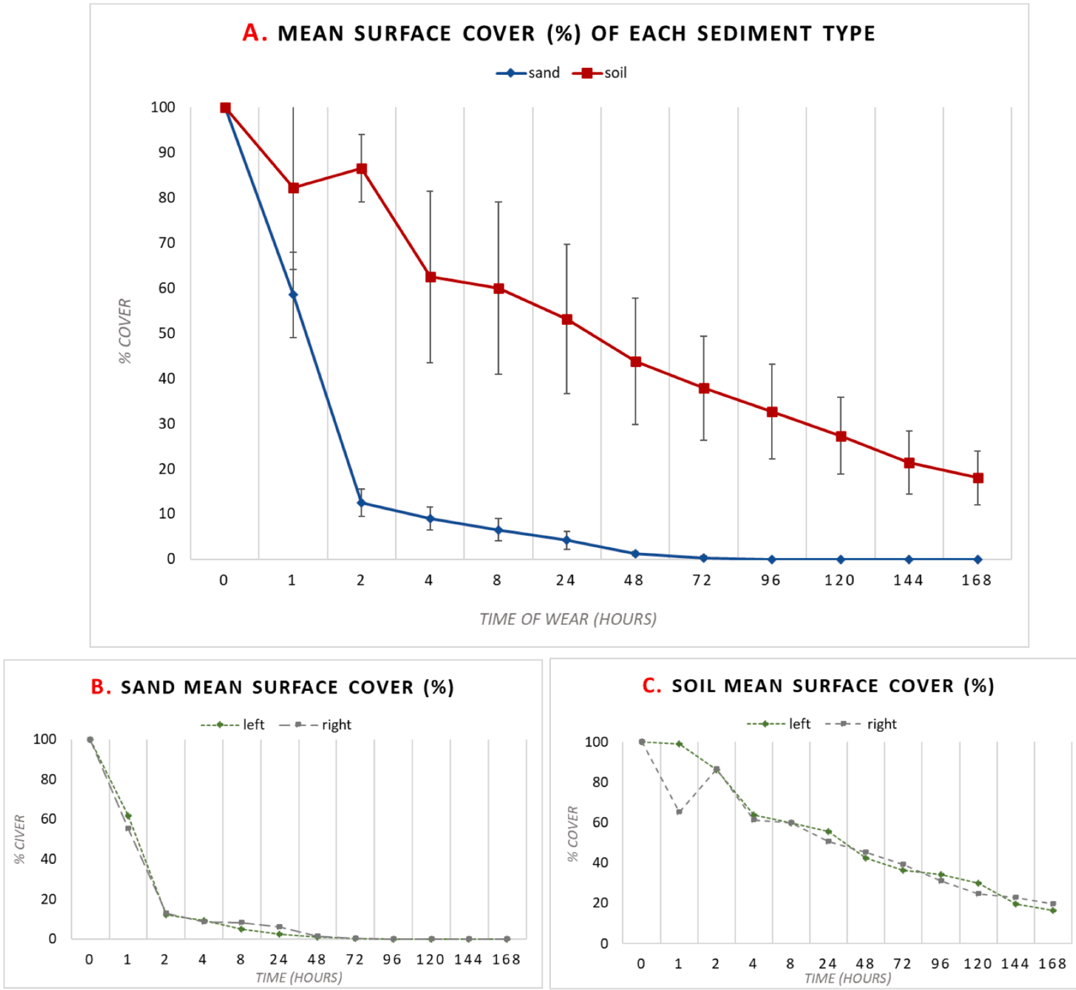



**Fig. 3.** The retention of sediment (% surface cover) on footwear following up to one week of wear in Experiment 1. (a) Overall mean ± standard deviation of loamy clay soil and dune sand retention (n = 6); (b) Mean retention of dune sand on the left and right boot (n = 3); c) Mean retention of loamy clay soil on the left and right shoe (n = 3).

**Table 2**
Initial quantitative assessment of true mass (g) associated with different levels of surface coverage (low, low-medium, medium, medium–high, high) for both loamy clay soil and dune sand on footwear. Mean values are provided (n = 3).

| | LOAMY CLAY SOIL | | | | |
|---|---|---|---|---|---|
| | Low | Low-medium | Medium | Medium-high | High |
| % Coverage | 8% | 23% | 54% | 69% | 91% |
| True mass (g) | 2.9 g | 5.2 g | 8.7 g | 17.4 g | 22.6 g |
| | DUNE SAND | | | | |
| | Low | Low-medium | Medium | Medium-high | High |
| % Coverage | 9% | 24% | 47% | 72% | 97% |
| True mass (g) | 1.3 g | 2.8 g | 5.3 g | 8.6 g | 15.2 g |

soil matrix characteristics, and the interactions between them [35]. These factors must be carefully considered during the exclusionary interpretation of environmental trace evidence in forensic reconstructions.

Significant differences identified in the persistence of loamy clay soil versus dune sand might be explained by the physical characteristics of each environmental matrix. Clay soil is typically more compacted, maintains moisture, and is much denser in comparison to beach sand which drains easily and is homogenous in its composition [36]. As such, loamy clay soil may be more readily preserved within the recessed areas of shoe soles, thus yielding a more abundant forensic sample for collection and analysis over time. A previous study by Morgan *et al* [33] identified differences in the characteristics of soil retained on footwear, with larger conglomerates lost more rapidly than silt-sized materials. This corresponds with the initial losses (0–4 h) but longer-term retention of loamy clay soil reported here (Fig. 3a). Furthermore, in their study of five different sediment types, Procter *et al* [9] identified that sediment type, and specifically moisture content, significantly influenced the extent of soil transfer to clothing. Similar findings are reported here in relation to persistence on footwear. No dune sand was identified beyond 24 h of wear (Fig. 3b), suggesting that such sediments may be of limited value when attempting to reconstruct links between persons and scenes of forensic interest. From our results, it is important to highlight that an absence of evidence does not negate that evidential transfer did initially occur [13,16].

Given that the two sediment types used in this experiment differed significantly in their retention dynamics, additional research is recommended to empirically assess a wider range of soil types, activity levels, and footwear options as in Procter *et al* [9]. Many variables are known to influence transfer and persistence, and, although this study accounted for several of those, further research is certainly warranted to explore the impact of additional factors on soil trace evidence dynamics [23,35]. For example, different footwear types may comprise a range of outsole materials, sole abrasions, and sole surface textures including flat and recessed areas and feature patterns. Subsequent empirical studies adopting a similar macroscopic analytical approach as employed here, should therefore seek to examine sediment transfer and persistence upon different footwear items including, for instance, training shoes and work boots.

In addition to temporal retention (% cover), our findings indicate spatial patterns in the distribution of transferred sediments across footwear surfaces. For example, loamy clay soil was more effectively retained in the arch (middle) and recessed areas of the shoe sole (Table 1); dune sand also persisted longer in areas with similar surface characteristics. Both sediment types were more readily lost from sole areas directly contacted with the ground. This corresponds with previous research findings, initially using plasticine proxies [27], prior to microscopic analyses of soil quartz grain surface textures [2] and fine sand fraction mineralogy [15,38] following multiple environmental exposures. The precise location of a persistent forensic soil sample has important implications for evidence recovery, analysis, and interpretation. The findings reported here recommend that traces from a pertinent forensic scene, are more likely to be retained in the arch and recessed areas of the shoe sole. Those areas should subsequently be prioritised for the collection of sedimentary evidence in support of more extensive laboratory-based analyses in forensic casework [38].

The experimental design presented has yielded valuable qualitative insight into soil distribution and retention (%) upon footwear. However, given that transfer and persistence is associated with the mass and size of individual and conglomerated soil particles [49], quantitative assessment of sample mass may also be pertinent to consider in relation to surface cover (%). Our results demonstrate a reduced forensic sample mass over time which corresponds with similar decreases in surface retention of visibly identifiable material (Table 2). Additional research should seek to fully explore the relationship between surface cover and the true mass of retrieved trace evidence samples over time, incorporating more extensive analyses such as soil texture and particle size distributions [27,49]. It is worth noting however, that detailed scrutiny of sample mass would involve an invasive sampling strategy, limiting the potential for successive analyses of % surface cover as presented here.

Although previous empirical study has explored transfer to clothing [9,28–29], this research is novel in that it applies a macroscopic approach to assess sediment transfer and persistence on footwear. The protocol described is non-destructive, cost-effective, and allows successive analyses of the same samples over time.

## 3. Experiment 2: Diatom persistence on clothing

Diatoms are a species-rich group of unicellular eukaryotic microalgae, commonly found in freshwater, marine, and terrestrial environments [4]. Diatomaceous earth is mined extensively for use in man-made products including food supplements, pest control agents, and throughout the manufacturing process (e.g., paint fillers) [39]. Subsequently, the prevalence of diatoms within our natural and artificial environments enhances the potential for these microscopic and chemically resistant particulates to transfer and persist as forensic evidence [40].

Published examples of diatom analysis in forensic casework are based primarily on the analysis of those species transferred from natural environments [48]; however, the value of diatomaceous earth as a forensic indicator has been realised since the 1970s when the material was used to insulate safe ballasts, providing a visual marker to identify assailants in burglary cases [40]. The existing research base has similarly primarily considered the forensic value of soil and freshwater diatoms transferred to clothing and footwear [4,7,11,13,14]. The dynamics of anthropogenic traces in the form of diatomaceous earth have not previously been empirically assessed. Although prior studies have all adopted microscopic approaches to analyse the species characteristics of individual diatom frustules, the availability of diatomaceous earth offers a useful visual marker for novel, successive, macroscopic analyses of diatom transfer and persistence over time.

This study aimed to assess the distribution and persistence of diatomaceous earth (% surface cover) transferred to denim, fleece, and acrylic clothing following 30 min, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h (one week) of wear. The impact of clothing positioning was also considered via comparison of diatom retention on samples worn on upper and lower parts of the body.

### 3.1. Methods

Ethical approval was not required in accordance with LJMU Faculty of Science guidance regarding observational work in the field. Consent was provided for data collection and sharing.

#### 3.1.1. Field protocol

Food-grade diatomaceous earth powder (DiatomPure®), comprising of ground diatom frustules, was used as a visual marker to assess persistence on new, unworn denim, fleece, and acrylic clothing (Table 3). The powder was carefully applied to 1 $cm^2$ subsamples [as in 7,11,13,14] of each fabric using a paintbrush contacted 5x with the respective swatch.

Three replicates of each clothing type were prepared for wear on the upper and lower body (Fig. 4). The subsamples were attached to clothing garments (same wearer) and worn during low-intensity daily activities (e.g., cleaning, studying, sitting) for up to 8 h per day. The samples were worn both indoors and outdoors, including a daily 3 mile walk in a residential urban area, during January and February 2021 (South Yorkshire, UK). The same individual (160 cm height) wore the clothing subsamples to ensure consistency throughout. The experiment continued regardless of weather conditions which included seasonal rain and wind.

#### 3.1.2. Data collection

Data was initially collected via a USB microscope (Jiusin) fixed in position at a $90^0$ angle, using 40x magnification. Four photographs, accounting for the whole surface area of the 1 $cm^2$ fabric swatch, were taken for each subsample at 0 h (initial transfer) and then 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 h post-transfer (total n = 936). All images were cropped using ImageJ and a 100 5x5mm square grid overlay was applied to each to facilitate calculations on percentage (%) cover. If any diatomaceous earth was present within each grid square, a positive result was recorded in red. If absent, a negative result was documented in black (Fig. 5). All four photographs were analysed to provide a total % cover for each subsample. Mean values were subsequently calculated from three replicates.

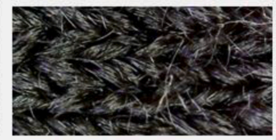
#### 3.1.3. Statistical analysis

The data met the assumptions for a three-way repeated measures ANOVA which analysed the combined main effects of clothing type (denim, fleece, acrylic), body position (upper, lower), and persistence interval (0–168 h), and any interactions influencing % surface cover over time. Sphericity was determined using the Mauchly test [47]. Effect size was reported as partial Eta-squared ($\eta 2$) and was used to indicate the variance accounted for by each factor. Significant effects were further analysed using pairwise comparisons with Bonferroni correction applied for multiple comparisons. Analysis was performed in SPSS v. 28 using a 95% confidence interval.

### 3.2. Results

Initial assessment of spatial distribution highlights the rapid loss of diatomaceous earth from the perimeter of each clothing swatch (Table 4). Although more material was lost from all three clothing types as the experiment progressed, initial differences in retention were identified with greater coverage on the denim samples over time (4–168 h) when compared to fleece and acrylic.

**Table 3**
Description and images (1x and 40x magnification) of the three clothing materials tested in Experiment 2. A USB microscope (Jiusun) was used to capture the 40x micrographs.

| Material | Composition | Surface characteristics | Photograph (x1) | Micrograph (x40) |
|---|---|---|---|---|
| *Denim* | *98% cotton, 2% elastane* | *Semi open weave, rough texture* |  |  |
| *Fleece* | *100% polyester* | *Open weave, rough texture* |  |  |
| *Acrylic* | *100% acrylic* | *Open weave, medium texture* |  |  |

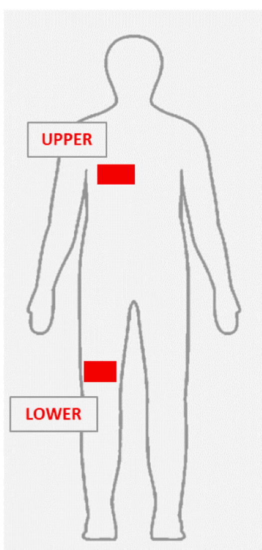



**Fig. 4.** The position of the upper and lower clothing swatches during the investigation of diatomaceous earth persistence on denim, fleece, and acrylic materials over one week in Experiment 2.

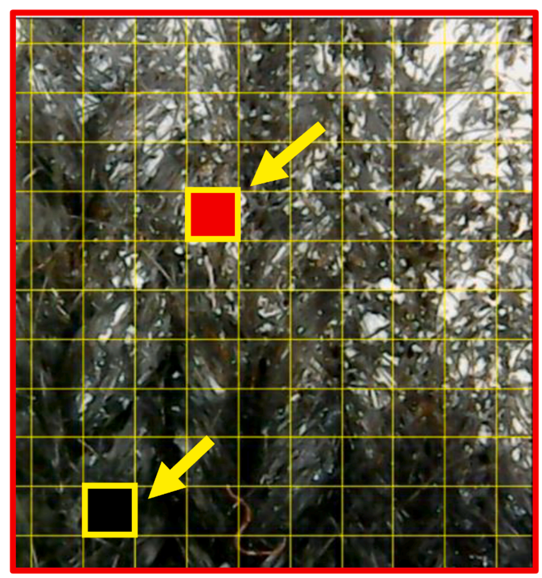
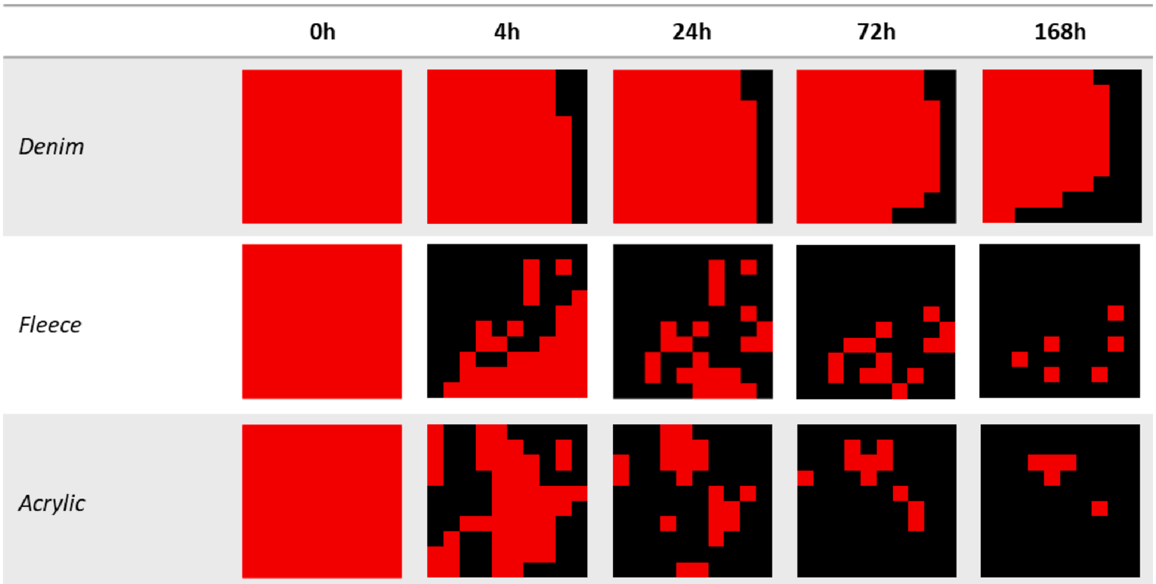


**Fig. 5.** Example of the 5x5mm grid overlay applied for the analysis of diatom percentage cover (%) on clothing using ImageJ in Experiment 2. The presence of diatomaceous earth in each individual square was recorded as a positive result in red; the absence of diatoms was marked in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Subsequent analysis of mean percentage cover (%) identified these differences more clearly (Fig. 6). The loss of material from denim was more gradual over time when compared to fleece and acrylic. For example, 60.4–62.1% of diatomaceous powder transferred to denim was visible after 168 h, compared to 10.5–16.6% (fleece) and 5.5–10.7% (acrylic). Decreases were relatively consistent between consecutive sampling intervals, with the most rapid loss of material occurring after 4 h of wear on the upper body (Fig. 6a) and 2 h of wear on the lower body (Fig. 6b). For example, the mean coverage of fleece decreased from 79.5% (4 h) to 66.9% (8 h) on the upper body and from 79% (2 h) to 51.6% (4 h) on the lower body. Relatively large differences in mean % cover were identified between sample replicates, with greater variability amongst all materials worn on the lower body compared to the upper region (Fig. 6).

Three-way repeated measures ANOVA found no statistically significant three-way interaction between clothing type, body positioning, and persistence interval on % surface cover, $F\,(24, 48) = 1.248$, $p = .252$. For the two-way interactions and simple main effects, a Bonferroni adjustment was applied. There was no statistically significant two-way interaction between clothing type and body positioning, $F\,(2, 4) = 3.564$, $p = .129$. A statistically significant two-way interaction was identified between clothing type and persistence interval, $F\,(24, 48) = 23.432$, $p < .001$, with a large main effect ($\eta 2 = .921$). The properties of the recipient clothing significantly influenced percentage retention of diatomaceous earth over time. Simple main effects identified that significantly more material was retained on denim than fleece or acrylic ($p = .002$, $\eta 2 = .911$). There was no significant difference between fleece and acrylic ($p = .253$) (Fig. 6). A statistically significant two-way interaction was identified between clothing position and persistence interval,

**Table 4**
Initial spatial analysis of diatomaceous earth transfer (0 h) and retention at 4 h, 24 h, 72 h, and 168 h on denim, fleece, and acrylic clothing in Experiment 2. Areas marked in red indicate the presence of diatomaceous earth whilst those areas in black indicate the absence of any transferred material.




$F(12, 24) = 5.932$, $p < .001$, with a large effect ($\eta2 = .748$). The location of a transferred sample influenced retention over time. Simple main effects identified that significantly more diatomaceous earth persisted on the upper body than the lower body ($p = .041$, $\eta2 = .424$) (Fig. 6).

Finally, persistence interval had a large, significant main effect on % retention over time, $p < .0001$, $\eta2 = .803$. Pairwise comparisons identified that significantly less diatomaceous material was present beyond the initial transfer assemblage (0 h) ($p < .05$). Although loss was broadly consistent, the percentage loss of material between consecutive persistence intervals was not always significantly different, especially in the later stages of the experiment. For example, whilst loss from 0 −0.5 h ($p = .002$ [± 2.5%]) and 2–4 h ($p = .012$ [± 11.5%]) was significant, that from 72 to 96 h ($p = .096$ [±5.25%]), 96–120 h ($p = 1$ [±2.08%]), and 144–168 h ($p = 1$ [± 2.07%]) was not (Fig. 6).

### 3.3. Discussion

This study is the first to investigate the transfer and persistence dynamics of diatomaceous earth on recipient clothing surfaces. The findings highlight that although percentage loss within and between the earlier persistence intervals (0–24 h) was statistically significant, retention was stable beyond 48 h of wear. Denim more effectively retained diatomaceous earth than acrylic or fleece, and clothing worn on the lower half of the body lost significantly more transferred material over time than clothing worn on the upper body (Fig. 6).

Our findings correspond with those of previous research exploring freshwater diatom persistence on clothing [13,14]. However, the loss of transferred material over time was far more consistent throughout this study (Table 4, Fig. 6). For example, Scott *et al* [13] demonstrated that diatom retention on different clothing materials varied over one-month of wear, with relative increases often observed between corresponding sampling points (e.g., from 1 h to 2 h). The contrasting trends reported here may be explained by the experimental approach which successively analysed the same fabric subsamples over time, rather than collecting and analysing separate $cm^2$ samples at each persistence interval [13]. Furthermore, Scott *et al* [14] previously identified variability in transfer and persistence due to diatom valve shape, size, and species-specific surface structures. The relative uniformity of the diatomaceous earth used here may therefore have limited temporal variability in this experiment [39]. The reported persistence trends closely align with those typically reported in the trace evidence literature (including fibres [34], pollen [12,33], gunshot residues [41], and soil persistence in experiment 1 [Fig. 3]) – an initial, rapid, statistically significant loss of material followed by longer term preservation of a less abundant assemblage (Fig. 6). This research therefore highlights that diatomaceous earth may be sought and retrieved as forensic evidence from clothing up to one week post initial transfer.

Clothing substrate had a significant influence on diatomaceous earth retention in this study, reflecting similar findings from freshwater diatom [13], pollen [8], fibre [34], and glass research [42]. Interestingly, previous work by Scott *et al* [13] identified a more abundant assemblage transferred and retained on acrylic, compared to eight other materials including denim. Acrylic was significantly less effective than both denim and fleece in the retention of a diatomaceous sample for macroscopic analysis in this study (Fig. 6, Table 4). This suggests that, although surface characteristics (e.g., an open weave, medium-rough texture) facilitate trace evidence entrainment and retention, differences may still exist between individual garments and the wearers of those. Further research is recommended to blend the macroscopic approach documented here with more extensive laboratory protocols, including microscopy [13,14], to quantifiably assess the preservation of individual valves from the diatomaceous earth matrix on clothing in line with different levels of macroscopic coverage. Such an approach may offer valuable insights into the identified differences between individual garment of the same general type.

Clothing placement had a significant impact on the retention of diatomaceous earth, with significantly greater persistence on samples worn on the upper body compared to the lower (Fig. 6). Although this is the first study to consider the impact of clothing location for diatom persistence, previous research has demonstrated a more abundant forensic pollen assemblage on lower clothing garments [19]. The findings presented here demonstrate the opposite in terms of abundance, however only small ($cm^2$) subsamples were worn in contrast to the whole garments reported in Morgan *et al* [19]. Further research is therefore recommended to consider a larger subsample area than the $cm^2$ area used here. For example, *Bull et al* [12] used 22x13cm swatches, Webb *et al* [8] employed 8.5x3cm test fabrics, and Procter *et al* [9] tested 40x40cm subsample areas in their analysis of soil and pollen dynamics. Such larger scale assessment in the context of diatomaceous earth transfer and persistence is especially warranted given that retained

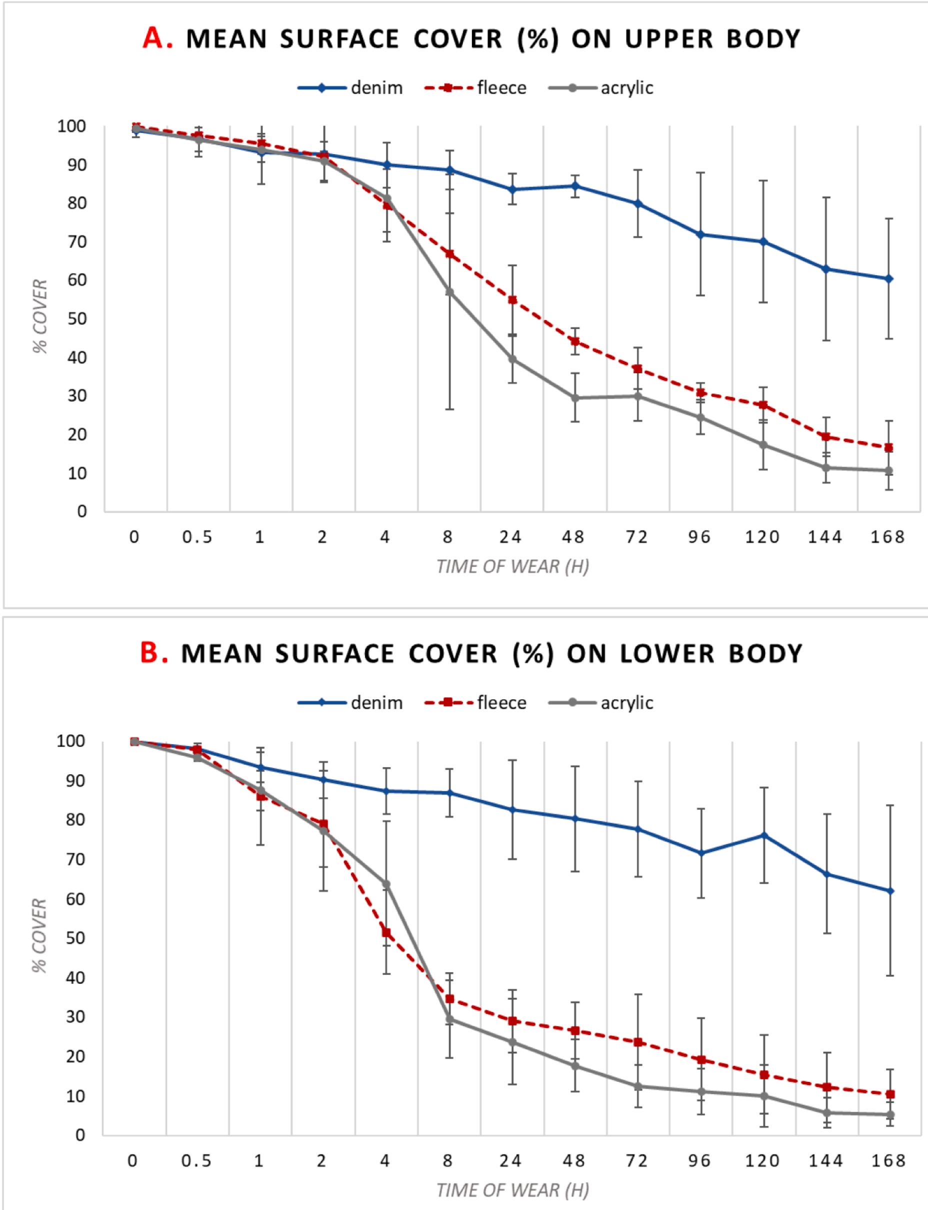



**Fig. 6.** The retention of diatomaceous earth (% surface cover) on denim, fleece, and acrylic clothing following up to one week of wear on the upper (a) and lower body (b) in Experiment 2 Mean values are presented (n = 3) with error bars highlighting the standard deviation between replicates.

material was consistently detected and identified using the macroscopic employed throughout this experiment (Table 4).

The use of visual markers, including UV and fluorescent powders, to explore trace evidence dynamics, is relatively established within the forensic research base [12,19,21–24]. This study is the first to apply diatomaceous earth in a similar manner for the investigation of environmental trace dynamics. Whilst this protocol offers a novel, non-invasive analytical approach, more extensive laboratory-based investigations may be recommended to consider additional complexities including the impact of species characteristics on evidence retention over time [11,14].

## 4. General discussion

Both studies generate promising insights, and highlight the value of a macroscopic approach, for qualitative assessment of environmental trace evidence dynamics. The findings presented have identified the impact of substrate characteristics, soil type, body positioning, and wearer gait on the transfer and retention of sediments and diatomaceous earth evidence to clothing and footwear surfaces in forensic settings. These findings are extremely promising and recommend subsequent empirical development to assess the influence of additional, diverse, factors including alternative soil and footwear types [9,7,11–14], seasonal comparisons [13,14,35], and the impact of secondary transfers [27,38] for visual analyses of pertinent forensic materials.

Macroscopic analysis via image processing may be particularly useful to explore the impact of activity level and wearer characteristics upon (environmental) trace dynamics in a novel and cost-effective way [20,35,37,44]. Our experiments incorporated relatively low-intensity daily activities, including walking, within the experimental design. Subsequent research may wish to explore the influence of different activity levels including, for example, moderate (e.g., jogging) or vigorous-intensity physical activities (e.g., running) upon the retention (%) dynamics of surface traces [23,35]. Additional development may also seek to incorporate a greater range of distances travelled and exposure environments to extend the findings presented here which involved 3- or 4-

mile walks in urban, residential locations. Furthermore, both experimental studies presented involved only one wearer; subsequent research should aim to incorporate multiple individuals to assess the influence of physicality (e.g., height, weight, sex) and gait (e.g., step length, walking speed, walking asymmetry, double support time [50]) on evidence retention. This may be especially important to consider in relation to footwear surfaces which are readily influenced by pressure, vertical, and frictional forces via direct exposure to contact surfaces [44].

Recognising that an initial transfer has taken place is an important first step in support of more targeted trace collection, analysis, and interpretation procedures [16]. Non-destructive macroscopic analyses incorporating image processing provide a useful approach to identify and visualise the distribution of transferred environmental evidence on recipient surfaces following up to a week of wear [28,29]. This may be particularly advantageous if the precise location of a transferred assemblage provides an indication of crime or post-crime activities which may be relevant for forensic reconstructions [19]. Whilst this, and previous studies [23], have demonstrated the value of visual analyses within forensic research, further empirical scrutiny to ensure reproducibility, generate reference databases, and to identify limits for interpretation, is required before application within forensic casework [10].

Visual analysis using an identifiable marker (e.g., sediment or diatomaceous earth) offers a straightforward, accessible, and non-destructive method to facilitate greater exploration of trace evidence dynamics [35]. This approach overcomes some of the limitations identified within the existing forensic research base, by facilitating, for example, consistent and successive analyses of the same sample area rather than the removal of individual subsamples for more extensive scrutiny [12–14]. Further research is recommended to build on and extend this forensic value. For example, technical refinement of image processing guidelines using ImageJ or Adobe Photoshop, may help to identify detection limits for different trace materials, and develop standards for comparable data to be generated by a range of forensic researchers. Such cohesion has the potential to generate diverse and usable reference databases which maybe applicable in a range of trace evidence disciplines.

Although structured studies exploring trace evidence dynamics are frequently reported [2,4,7–9,11–15,17–19,21,27–29,31–34,38,42], recent commentaries have identified the need to incorporate new technologies [43], materials science perspectives [44], and a universal experimental protocol within future transfer and persistence research [23]. Our study has initially demonstrated the advantages of using image analysis to explore the retention and distribution of soil and diatomaceous earth on recipient surfaces, supporting prior calls for technological innovation [39]. Our findings have demonstrated the value of a general, widely applicable, macroscopic approach to assess spatial and temporal trends within environmental trace dynamics, corresponding with recommendations for a universal experimental transfer and persistence model initially proposed by Ménard *et al* [23]. Our protocol offers a discipline-independent framework to empirically reconstruct and interpret trace evidence dynamics. We demonstrate that material properties including those of the recipient surface (e.g., footwear tread, clothing weave), the trace material (e.g., moisture, mass, uniformity), and interactions with contact surfaces, influence the spatial and temporal distribution of environmental trace evidence within recipient surfaces post-transfer [44].

Finally, visual analysis via image processing offers a cost-effective, non-invasive, and widely accessible approach to support forensic research within trace evidence dynamics [23,9]. The findings from this research demonstrate that macroscopic approaches contribute important data, and insight, to the forensic evidence bases which frequently underpin trace collection, analysis, and interpretation strategies [16]. Our experimental methodology represents an innovative and accessible way of pursuing research within the forensic sciences. This is particularly important in support of alternative curriculum design and teaching methods within forensic science education [45] and to enable the representation and inclusion of diverse groups within the forensic research community [46].

## 5. Conclusion

In summary, the experiments presented here demonstrate the novelty and value of using a macroscopic approach, incorporating spatial and temporal analyses of photographic images, for the assessment of environmental trace evidence dynamics. Although soil type, clothing type, and body positioning significantly impacted soil and/or diatomaceous earth distribution and retention, a transferred assemblage frequently remained identifiable within and beyond one week of continuous wear. Recognising this initial presence provides a scientific basis to support more extensive laboratory-based investigations of transfer and persistence in subsequent research. The data and information presented throughout this paper ultimately highlights the value and increasing potential that macroscopic analyses offer for an empirical assessment of trace evidence dynamics using novel, non-invasive, and widely accessible technologies.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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